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UNITED NATIONS

Bulletin on NARCOTICS

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DIVISION OF NARCOTIC DRUGS

Geneva

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Progress in Iran: The struggle against the evil of narcotics up to 1960

By A. E. Wright, C.M.G., O.B.E., F.R.S.A., Technical Assistance Expert in Iran

To appreciate the current law on narcotics in Iran and the process of thought and action that has led up to it, it is essential to understand something of the background of the problems with which the legislation deals. Law cannot be treated in isolation: it is the expression of a national point of view of a social structure and a climate of opinion, besides being the basis of action within the national administrative structure, in order to deal with a set of problems. Narcotics problems are general, in that they have a similarity of outline in all countries that have attained a certain level of social development. They are particular in that they must be dealt with within the boundaries of a nation in accordance with that nation's own genius and in accordance with that nation's own views as to the correct approach to its problems. In the field of narcotics, much that is general has been laid down by the League of Nations and the United Nations in the various international treaties for which they have been responsible. Iran has accepted the general outline of the proposed Single Convention on Narcotics, and has incorporated many of the provisions contained therein in the law on narcotics that was passed by the Iranian Parliament in June 1959. But Iran has had to judge how far the country could go in the general directions indicated, and what special conditions had to be met by special terms in the legislation. Even language presents a difficulty: Farsi is a language that goes back to and derives from a history of culture, thought and institutions wholly different from those which are embodied in most western thinking on problems of legal drafting and form — to say nothing of the substance of the laws.

In general, then, narcotics legislation has four stages. In the first, paradoxically enough, there is no legislative control over the production and use of narcotics in a country. There may be social pressures and customs; but there is at this early stage no legal enactment that seeks to control or regulate the production or use of narcotics. It is not even clearly realized what substances are narcotics. At the second stage, there is control for fiscal or trade purposes. It has been realized that certain substances are valuable and that the state can profit from their use: without in any way thinking whether trade in them is good or bad for the consumer, trade is fostered and taxes are levied on the substance or on the trade in it.

At a third stage, there is control for sociological purposes combined with or deriving from the control which has been established for the fiscal or trade purposes already mentioned. This type of control will be restrictive, and it normally uses fiscal means in order to be effective. The government concerned has been told or has realized that the substances with which it is dealing are dangerous to the community; or at least that their unrestricted consumption may have effects that are either not easily calculable or that are represented as being actually harmful. A country that has reached this stage is mature: its government has progressed from the stage at which it merely thinks in terms of revenue or profit, to that at which it thinks in terms of welfare. The view that high taxation as a restrictive measure can be an end in itself in this field has been widely held. Where the ultimate end of the government is restriction, the operation of the law of diminishing returns in respect of the tax yield is accepted as a natural consequence of the basic policy of the government. But the operation of this law and the feelings of a government which is suddenly faced with the reduction or the complete loss of a certain tax yield render this conception a hazardous one. Experience of taxation of tobacco and liquor, for instance, shows that an increase in tax rates may be followed immediately by a reduction in consumption; but after the consumer has accustomed himself to the shock of an increase in price he frequently goes back to his former rate of consumption, and the tax yield goes up. Narcotics control, therefore, at this stage of development usually consists in a combination of high taxation and statutory controls on production and distribution.

Thus, full national maturity in the sociological sphere accompanies and conditions a decision to legislate so as to prohibit the production and consumption of a narcotic substance that is accepted internationally as dangerous to the consumer. I am not referring to drugs that are accepted as necessary for medical purposes; I am referring to drugs that are regarded as dangerous by informed international opinion. Such a view, as an expression of a point of view, in theory, is a long way from the actual national outlawing of the drug or drugs by legislation and by enforcement that is reasonably effective. A country which takes the bold step of prohibiting a narcotic or narcotics is advanced in its sociological thinking, and it takes a calculated risk in the effects that may be expected from such a prohibition, when the prohibition is not internationally effective. Thus, to be effective in the country, the prohibition must be accompanied by an effective stoppage both of imports and local production. The medical and economic needs of the country must be calculated, sufficient drugs must be available for medical needs, and an alternative employment must be available for persons whose livelihood disappears due to the prohibition. It is axiomatic that a prohibition, to succeed, must have the support of public opinion.

It must be taken for granted that no enforcement will be 100% effective, and that, therefore, although a good enforcement system is an essential ingredient in a national prohibition, other means must be sought to make the prohibition as effective as possible. Apart from public opinion, which will destroy the prohibition in time if it is not generally favourable, there must be long-term planning in the medical and economic spheres; and there must be an attack on the basic causes of the

tendency to use a drug or drugs which have led the national government to introduce a prohibition. While, therefore, immediate measures are essential, and while, in the early stages of the administration of a prohibition, there will be a natural emphasis on the enforcement of the law, if the measure is to have lasting effect the government must think in terms of basic causes and long-term plans.

When we apply these considerations to Iran, there is an indication of an important cause of the use of narcotics in Iran since the war in my article in the United Nations *Bulletin on Narcotics*, vol. IX, No. 2, where I gave poverty as the cause which must be eradicated if there is to be full success in the campaign against drugs. It follows that all measures which tend to raise the standard of living will work against the drug habit, in so far as that habit is endemic among the middle classes and the poorer people. Apart from the effects of poverty and the consequent lack of medical facilities, in Iran a tendency to abuse drugs generally in society was due to the social and economic strains which followed from the Second World War and the rapid modernization of the state. Medical measures are being taken and economic strains are being lightened, so far as the means of the country and of those agencies that are helping it will allow. In general, public opinion is in favour of the prohibition, and enforcement is strong. It will be seen that much of the work that is being done, a great deal of which implies recourse to legislation on land tenure and taxation, for instance, has little to do directly with the anti-narcotics legislation, which is the specific subject of this paper. But it must be understood that in Iran the ramifications ot the problem, which the country decided in 1955 could only be met by a prohibition policy, are such that a wide preliminary view is necessary, in order fully to appreciate the special laws on narcotics passed since the war.

The progress of legislation, again, must be judged by the progress of the problem. There is nothing static in this field. Strong enforcement of the 1955 law, which was revolutionary in concept, has reduced the use of opium against which this law was specifically directed, by three-quarters. But the distribution of the habit was so widespread that even such a reduction has left a considerable residue. And the rise in price of opium and the demand for narcotics has led to a recourse to heroin, and in a lesser degree to morphine and cocaine, which introduced a change that is both as dangerous as the old recourse to opium, and as difficult to deal with even from the limited point of view of law enforcement. While, therefore, the present is a useful time to sum up the progress that has been made in legislation in order to deal with the manifold problems which abuse of drugs implies in Iran, it is not suggested that the position is static, or that further legislative steps may not be needed in the future. Iran has reached a peak at the moment: the nettle has been grasped, the implications of the problem have been widely studied, and a step in legislation has been taken which will mark the height to which Iran has attained. But there are continuing problems and new problems, and there is little doubt that in the future further legislation will be attempted. At present, the process has gone as far as is needed and as far as can be expected.

Sociological legislation of the type embodied in the two Iranian laws of 1955 and 1959 (see *Bulletin on Narcotics*, vol. VIII, No. 3; vol. XI, No. 1; and vol. XII, No. 2) must derive from an advanced social consciousness in the society that imposes such laws on itself, and from an advanced view of the purposes of legisation in this field. A country may well view with apprehension the possible consequences of such laws and it may wonder whether or not it has the means to make them effective; but the basis of the conclusion that such legislation must be attempted and that the effort to make it effective must be made (whether alone or with outside help is not immediately relevant) represents in responsible circles in the country an appreciation of social issues which is the mark of an advanced social mentality. Parliamentary legislation on opium in Iran began with a ban on its non-medical use in 1910, not many years after the passing of the Constitution. In 1928 a government monopoly was instituted (following the visit of a League of Nations Commission of Enquiry in 1926, to which a further reference is made below). It was intended to follow up the institution of the monopoly with a complete ban on the cultivation of the opium poppy at the end of 10 years. In 1938, the cultivation of the opium poppy was duly forbidden, but not completely in 25 districts. In 1946, after the Second World War, a movement was set on foot to forbid the cultivation of the opium poppy completely, but the time was not ripe, and only a limitation of cultivation was approved. It is thus evident that Iran was aware of wider issues. The question before the League of Nations commission which visited Iran in 1926 was whether the production of opium could be restricted to medical requirements, with the corollary that the smoking of opium should be stopped. Thus the sociological issue was already being faced, together with the fiscal and economic problems. The report of the commission is a classic of its kind. The conclusion that was reached was that Iran could ban the smoking of opium and could restrict the production of opium to medical needs without damage to the economy of the country. It was possible to replace the opium poppy in the agricultural economy of Iran. Methods were suggested by which the impact of the restriction that was proposed could be minimized. In fact, as we have seen, the control over the production of opium that was envisaged in this period never succeeded. Many circumstances proved to be too much for the intentions of the governments of the time.

There was therefore a continuing crisis. The Second World War and the difficult times which followed the war brought the crises to a head. After the attempt to deal with it in 1946, it finally erupted in 1955, when the first law prohibiting opium was passed by Parliament. The opium habit was socially reprehended by an informed and vigorous minority, whose views were strongly represented by the Anti-Opium League. Its consequences upon the morale and upon the development of the country were being recognized. The issue which was now faced was not that which had been before the League of Nations commission 30 years before. It was that the health and morale of the nation were being undermined by the habit of smoking opium. The issue was now social rather than economic. The campaign of rehabilitation could not succeed,

however, without legislation which would embody what was now felt to be the only possible solution to the problem. Regulation and restriction had been tried and had failed. The issue had to be faced, as it was vital for the continued life of Iran as a nation. The conclusion which was reached was that the only solution was to cut out the cancer — a prohibition of the growing of the opium poppy and of the use of opium in any form was essential. Parliament had to be convinced of the absolute necessity of legislation to this end, and a means had to be found of making it effective.

The placing of legislation on this issue before the Iranian Parliament in the autumn of 1955 was a test of the strength of the Parliament and its ability to grasp a basic sociological issue. Social and educated opinion were only partly indoctrinated against the opium habit. A good deal of money was made from it, and a strong administration would be needed to make the proposed prohibition effective. The work of the Anti-Opium League in printing pamphlets and in the use of other means of propaganda and education was forceful. The presentation of the draft law in Parliament lacked nothing of drama and vigour. Against the very strong measures that were proposed were the fact that regulation was the normally accepted method of dealing with the problem; prohibition had failed elsewhere; there would be a loss of income to the state; there would probably be a loss to agriculture; there was still not a unanimous condemnation of the habit of smoking opium. In favour were the realization of the difficult situation that faced the country; the psychological impact of the dramatic decision to propose a prohibition; the view that in Iran a prohibition could succeed if it was accepted generally as necessary, and if international opinion were mobilized in its favour; the view that the economic effects of such a prohibition on the agriculture of the country might have been exaggerated, and that in any case they could be overcome; and that, in general, social pressure could be built up against the habit to an impressive degree.

The law which was passed embodying the prohibition was reproduced in the *Bulletin on Narcotics*, vol. VIII, No. 3, in an article by Dr. Saleh, the Minister of Health at that time. It was a short Act, consisting of five articles and one note only. The regulations, which contained the detailed provisions both for penalties and for an administration, were passed later. A further reference to the administration of this law and to its effects is to be found in my article in the *Bulletin on Narcotics*, vol. IX, No. 2, already cited. There is thus no need to go into details in this paper. The law stated the prohibition and the duties of the Health Ministry and other ministries; most of the detailed effective legislation was in the regulations, a point to which we shall have to return.

A critique of this law will emerge from the considerations that led to the drafting of the new, expanded law of 1959. But the fact of criticism and the decision of the Government of Iran to remodel the law must not blind us either to the revolutionary nature of the law of 1955 or to its merits. In December 1957 I wrote of it: "This law is complete in conception and has so far served the drive against opium so well that it is proposed that any fresh legislation should be a continuation, amendment and sequel to the law of 1955; piecemeal legislation should in any case be avoided in dealing with so difficult and controversial a subject as opium. There should be no great difficulty in securing the passage of a new law which is the logical sequel and complement to one which has had so great a measure of success; which, whatever may be the faults in detail which have emerged from two years' administration of its provisions, was so well conceived as a complete view of the whole field which the drive against opium would have to cover. The law itself provides for a review of its provisions for an administrative organization early in 1959, a suitable time for a general revision. A point to be noted is the essential unity of the present law. I cannot too strongly emphasize the need for keeping this subject in proper focus: the main problems are health, sociological and economic problems; the proper ministry to deal with them is the Health Ministry. The law of 1955 was, in fact, so revolutionary in what it attempted to do, and it touched both the life of the people and the working of the administration in Iran at so many points that it was inevitable, under a consistent policy, that there should be a heart-searching after a suitable interval of time, and that a revision of the law should then be placed before Parliament without undue delay. There were special points in the anti-opium drive in Iran: the lack of a proper definition of addict; the lack of good statistics; the extent to which the opium habit was found not as true addiction but as a habitual use among the working population; the link of the habit with poverty, and, for these reasons, its sociological and medical importance; the need for education of the public and the need for a further study of law and administration in general, apart from the peculiar problems of the Iranian case, all of which led to the correct conclusion that after a limited period there should be a new law, based on accepted international principles and on a further study of Iran's own problems in the light of those principles.

Study of the law was being undertaken in the Ministry of Health, which had been the sponsoring ministry for the 1955 law, throughout the first three years of the operation of this law. Study was also undertaken by the United States overseas missions and by a United Nations mission. The Minister, now Dr. A. H. Radji, therefore had before him not only the fruits of the work of the Ministry, but also the results of study by two outside agencies, in deciding on the form and content of a new law, and of a revised administration.

Thus, it was proposed to widen the scope of the law to include all narcotics. It was necessary to improve the definition of and the punishment of offences and further clarify the position of government officers and public bodies that might be concerned with narcotics offences. It was desirable to introduce more orthodox financing, while agricultural measures were left to the Ministry of Agriculture, which has already drawn up a project for a new bill that would cover its work in this field in so far as legislation was needed. Naturally, the Health Ministry accepted the basic positions of the 1955 law, especially that the problem was one for the Ministry of Health, with the co-operation of other ministries, and with improved general co-ordination. A further proposal was considered,

however, for a new approach that laid the greatest stress on enforcement, and purported to create a new department for this purpose under special regulations, and not under the control of the Ministry of Health. This proposal was supported, but was eventually not accepted. The Government stood by the principles of the 1955 law.

Lastly there was a proposal for a consolidation of the law on narcotics based on the views embodied in the draft Single Convention, of which the second and third drafts became available while the new legislation was under consideration. The Government did not attempt a consolidation — indeed, it is certain that the time is not yet ripe for such a step — but it did introduce a number of provisions in the law which was eventually passed by Parliament, which derive from United Nations thinking on narcotics problems. This law, enacted in June 1959, also embodied the use of the 1955 law as a starting point, the remodelling of the administration under one high officer of government, and the inclusion of all drugs under one legal system, for instance. Further points in the new law which are the product of serious thought and international study and consultation are the giving of statutory authority to the state import monopoly of drugs; the improved definition of offences, and the clearer statement and the more severe incidence of penalties; the improved provisions for the care of addicts, with special provisions for finance for this need; the setting up of an administration which not only has statutory authority, but also has improved powers to co-ordinate the work of the campaign under the control of the Minister of Health; and the grant of a rule-making power to the Minister in respect to the issue of lists of prohibited or restricted drugs. For a more detailed analysis of the law of June 1959 by Dr. A. H. Radji (the present Minister of Health, who placed this Act before the Iranian Parliament), a reference may be made to the *Bulletin on Narcotics*, vol. XII, No. 2.

This legislation is to be further developed by a brief bill, now under consideration by Parliament, which will carry further two tendencies that are strongly marked in modern Iranian legislation in this field. The first is an extension of the offences for which the death penalty is provided. In the law of 1959, this penalty exists for repeated offences of smuggling or manufacture of prohibited drugs. In the proposed new law, the death penalty will be extended to include any offence of illicit importation of prohibited drugs. The deterrent effect of stringent penalties is recognized. The second is the tendency to widen the scope of the law to catch the financiers and organizers of smuggling and drug trafficking. In the 1959 law, it was made an offence to aid drug traffickers or to profit from certain forms of trafficking. In the proposed new law, the Minister will be given the power to investigate the sources of the wealth of any person living in a frontier area where such wealth appears to be unusual in relation to the ostensible source of income of the person concerned. This proposed law is, as I have indicated, an extension of the 1959 law and not a new departure, since it carries to a logical conclusion existing tendencies that are strongly marked in the existing law. The present position in legislation in Iran is the culmination of the period of sociological legislation that was marked down as a general characteristic of a fully developed social conscience in a nation. It is difficult to predict future developments. They would depend on the development of the continuing problem represented by the narcotics traffic and the attempt to get rid of it. They would depend as much on world affairs as on Iran itself; and on developments in the field of international control. It is enough for the purposes of this paper to sum up the position in Iran in the terms which were laid down earlier as characteristic of national narcotics legislation, without attempting to predict the future. But before making a final summing up it is necessary to remember an important aspect of the general picture to which Iran has given a great deal of attention in the immediate past, in respect of which there are important provisions in the law of 1959. I refer to the setting up of courts which will interpret and administer the penal provisions of the law, since without such courts no legal system can be more than an academic exercise in law-making, or a capricious exercise of power.

To sum up progress in Iran, regulation did not succeed, and was not regarded as an answer to Iran's problem in this field. Prohibition was successfully introduced in 1955, when prudence might have suggested that half measures were more likely to be acceptable. Learning by experience of the working of prohibition, however, Iran has now introduced legislation that would be regarded as advanced in any country in the world. It has a wide scope; it sets up a new controlling administration on the lines suggested in the draft Single Convention; it introduces and enforces more stringent penal provisions; it has improved the working of the courts; it has greatly improved financial working, a reform without which any legislation that purports to set up an administration is doomed; and it makes adequate provision for the cure and care of drug addicts, within the means of which the country disposes, a movement which will be carried further as a result of studies which are now being made of the rehabilitation of dtug addicts as well as of the most up-to-date methods of their cure. The further law which is proposed, described above as an extension of the law of June 1959, not only further strengthens penal provisions; it also carries a step further the determination to carry the war into the camp of the financier and organizer of the large-scale smuggling traffic across Iran's frontiers, which is now regarded as more of a danger than the illicit cultivation of the opium poppy in Iran.

I have already stated that I do not propose to attempt to prophesy as to the future. But I reaffirm the position already indicated that internal legislation in Iran in this field has now reached the end of a stage of development. Internally, further developments will depend on whether and to what extent there is a demand for consolidation of the law, and what demands arise in the field of humanitarian work in the field of drugs. Much will depend, again, on the effectiveness of the administration of the present law. Good administration depends on factors that are quite outside the field of legislation and cannot be created by legislation. Here we can only note the good that has been done, and trust that equally good results will accrue in future. It is probable that the greatest effect on Iran's problem in the near future will rest in the field of foreign relations. The supplying of narcotics illicitly from outside the country has to be stopped.

Cannabis as a medicament

By J. Kabelík, Z. Krejčí & F. Santavy

The Institute of Hygiene and Epidemiology (Director: Prof. Dr. J. Kabelík) and the Institute of Medical Chemistry (Director: Prof. Dr. F. Santavy) of the Medical Faculty of the Palacky University, Olomouc, Czechoslovakia

Introduction

Within the scope of a systematic study of antibacterially effective substances (5) present in the flora growing in the temperate climate of Czechoslovakia, we have undertaken to investigate more than 3,000 different plant species. In this way we came upon the Indian hemp plant — *Cannabis sativa* var. *indica* — which has been investigated in detail. The subsequent finding of the antibacterial effect of the extract from *Cannabis indica* — direct evidence was provided some years ago at our Institute of Hygiene (46) — stimulated much interest in cannabis from the medical point of view, and resulted in numerous reports published from different institutes and departments (36, 43, 49, 51, 65, 78, 81, 82). Independently from us the antibacterial effect of the extracts from *Cannabis sativa* was described and studied some years later, particularly by Ferenczy (24, 25) and Drobotko (20). The interest of our clinicians was aroused in this respect by the very good results achieved with the antibacterially effective substances from various plants referred to in the literature (22, 23, 29, 59, 73, 74, 86, 87 [1]).

The preliminary results obtained were published in 1952 in *Lèkarské listy* (47) and in a compilation of communications submitted at the scientific conference held by the university in Olomouc in 1955. A survey of our work was published in the journal, *Die Pharmazie*, 1957-1959 (41, 50, 52).

Our paper is divided into the following sections:

1. Treatment with cannabis in ancient, folk and official medicine up to the beginning of the twentieth century.
2. A brief survey of the methods of isolation and the physical and chemical properties and structures of the isolated antibacterial substances.
3. Methods and results of the bacteriological experiments.
4. Survey of clinical experiences.

1. Treatment with cannabis in ancient, folk and official medicine up to the beginning of the twentieth century [2]

By J. Kabelík

The knowledge of narcotics and stimulants extends far back to ancient and medieval times but since then, as far as is known, no new discoveries have been made. In Europe, of course, some of them appear not to have been known until later, as was the case with the narcotics of the Old World — for example, with hashish (44, 88). Europeans learned of it for the first time through the Crusaders, but its deliberate use as well as abuse — according to Perrot (67) — came from Napoleon's soldiers, who introduced it after their return from the Egyptian campaign.

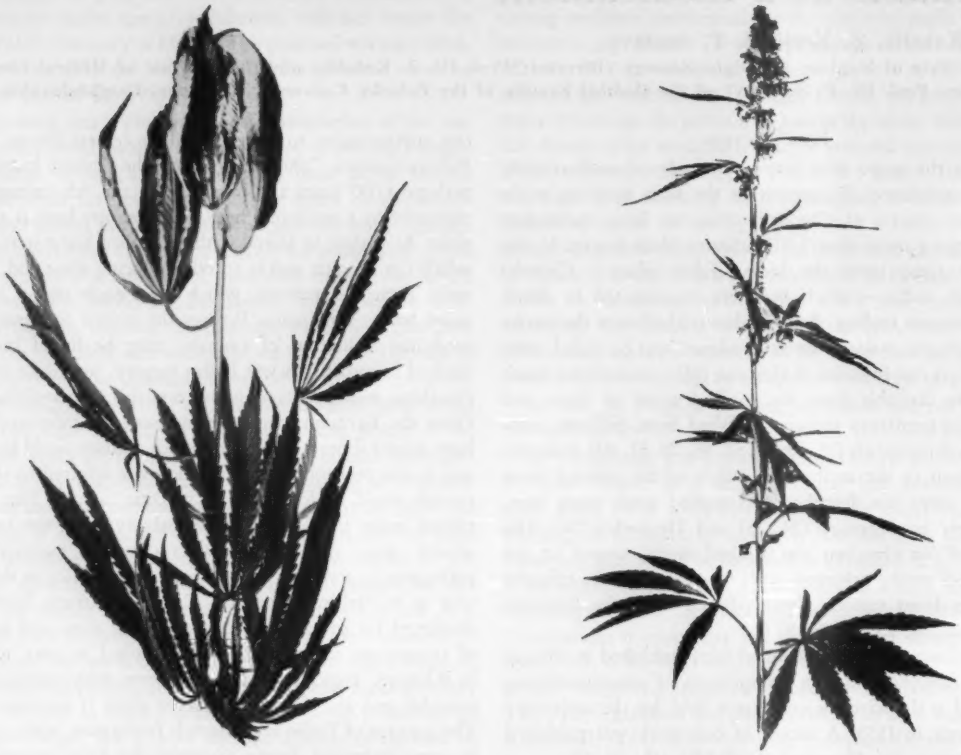
Cannabis is, however, a very old cultivated plant apparently indigenous to central Asia (68). Cannabis was found by our archaeologists to have existed in central Europe in the Bylony Culture (7,000 years ago). The Chinese knew of it perhaps 4,000 years ago, certainly in the 9th century B.C., primarily as a medicinal herb and a century later as a textile plant. According to Herodot, the Scythians knew it as a plant which can be spun and as an oil-producing plant and, apparently, even as a narcotic which they made use of in their steam baths — the sauna. Perhaps the earliest accounts of the medicinal utilization of cannabis may be found in Indian medical literature. Ancient Indian surgery, according to Susrat (Samhita), used hyoscyamus and cannabis as anaesthetics (32). From the Egyptian medical papyruses (64), information has been gained about a plant from which cordage could be made, and it was probably cannabis which was referred to. But no records could be found on its narcotic action. The preparations made from it (in all probability from the cannabis shoots) were applied externally — namely, exclusively as antiseptics — and then perhaps even as analgetics, in the same way as in Hellenic medicine. Cannabis extracts have been employed for irrigation in diseases of the anus, and in form of compresses the drug has been applied to sore toenails. In Rhamses' papyrus, washing sore eyes with extracts from cannabis and also from some other plant is recommended. The papyrus of Berlin recommends fumigation with cannabis in some undefined disease. Cannabis has been prescribed in feverish diseases of the bladder and, even at present, in homoeopathic medicine it ranks first when cystitis is treated. Furthermore, extracts combined with honey were injected into the body of the uterus to achieve constriction of the uterus and, externally, an ointment combined with fat was applied antiseptically in the same way as was done in medieval medicine up to the present time. Cannabis shoots were well known to Galen (27) and to Dioscorid (85). Homer's nepenthes — potion of oblivion — has been identified by some authors as a cannabis drug, but generally it is believed to be a preparation made from *Hyoscyamus muticus* — a plant familiar to the Egyptians. Both these authors make greater use of the seeds and of the oil extracted from them than of the cannabis shoots. It is the same in old popular and even in modern medicine. The seed pulp was a favourite dish, and from the seeds an edible, industrial and medicinal oil was obtained. In Czechoslovakia, a preparation from seed pulp was recently introduced by Šírek (60) to act as a roborant diet in treatment of tuberculosis.

But hemp seed does not form the subject matter of this paper; it is only treatment with cannabis shoots which is discussed, and only with regard to the healing of wounds, and not to the hashish effect.

The uses mentioned in the Egyptian papyruses point fundamentally to antiseptic use. Analogous uses were known, in varying degrees, to African natives (28, 29), and were recorded

[1] Comprises an extensive survey of reports by authors from the USSR.

[2] The botanical-historical and technical aspects of cannabis are not dealt with in this report.



1 a — Female plant. 1 b — Male plant.

FIGURE 1
Cannabis sativa var. indica

in medical herbals. There is no information on the narcotic action. It is of interest that in Egypt they are supposed to have learned of the hashish effect only during the Middle Ages, from the Arabs. This could be explained by the fact that the Arabs were the first to import the variety producing the physiologically active resin, for at present *Cannabis indica* Lam. is not considered to be a species but a variety of *Cannabis sativa* L. and not even a particular variety, Cannabis is a very variable and plastic plant of variable height and variously membered and formed leaves; there exists a monoecious variety, too. Any cannabis plant can produce hashish under favourable climatic conditions. Vice versa, according to Pulewka (71) the Indian variety does not always produce the narcotic substance, not even in warm countries. Plants cultivated in 369 places in Anatolia did not produce hashish substances throughout; the occurrence was conditioned by the climate and the habitat. Likewise, the hashish effect has not been found present in *Cannabis indica* cultivated in north Moravia, though this variety grows exceedingly well. The stem attains a height of about 4 m, bears rich seeds, and the tops produce resins which have a very pronounced antibiotic and analgesic effect. It is possible, however, that there was no hashish-producing variety grown in ancient Egypt, for the climate was, at that time, in all probability rather damp and subsequently much cooler than it is at present. This may be concluded from the fact that in antiquity in Italy and the Balkans snow was a more frequent phenomenon than it is nowadays, and north Africa was the granary of Rome, and not a desert.

All the information obtained from European folk medicine with regard to treatment with cannabis shows clearly that there do not appear to be any narcotic substances in it, or if there are then only in a negligible amount. Instead of that, emphasis has been laid on the antiseptic effect, hence on the antibiotic and to a small extent even on the analgetic effect, which has been upheld in official medicine up to the beginning of this century. In Austria, up to World War I, a salicylate collodion combined with *Extractum Cannabis* was prescribed for application to corns. Unna's green salve, which was used as a remedy for lupus, contained: *Acid salicyl.*, *Liq. Stib. chlorati* āā 2.0, Extr. *Cannabis ind.*, *Creosoti* āā 4.0, *Adeps lanae* 8.0. It may be noticed that the substances contained in cannabis have a powerful antibiotic effect upon Myc. tuberculosis, as will be discussed later. On the basis of the results obtained from our investigations, it is suggested that in many analogous

cases it would be advantageous to return to cannabis preparations again.

The ancient herbals (17, 63, 85) and those of the Middle Ages mentioned medical use of the seed, the roots (emoelients) and of the tops containing resinous substances. As previously stated, only the latter will be discussed here in detail. The leaves and the juice extracted from them or the macerated leaves were used as a vermifuge for horses, and fisherman soaked the ground with a liquid prepared from them to force up the dew-worms, which they used as bait. Tabernaemontanus (85) and Kramerarius (63) recommended kneading the dried leaves with butter and application of them in form of an ointment to burns. We have obtained positive results with extracts from cannabis in treatment of burns. Ruellius (cit. 63, 85) advised the use of cannabis extract as ear-drops in the treatment of ear ache, and also for treatment of wounds and ulcers. Women stooping due to a disease of the uterus were said to stand up straight again after having inhaled the smoke of burning cannabis. In cystitis and in urinary diseases, a decoction of hemp shoots with wine and water was recommended; the steam was allowed to rise as hot as could be endured against the perineum, after which the patient was advised to urinate. This use has more to do with the analgetic than the antiseptic effect. As previously mentioned, homoeopaths value highly both the teep cannabis D_2 à 0.25 g (teep is the fresh drug ground with lactose) and the *tinctura Cannabis indicae* D_3 up to D_4 which are employed in cases of cycstitis and of urethritis. The homoeopathic utilization of cannabis is fundamentally based on its effect on the central nervous system — i.e., in migraine as discussed in Schoeler's *Kompendium* (76) and by Auster & Schäfer (9). The homoeopaths do not otherwise utilize the antiseptic effect when they employ it internally; it is only the centrally sedative action they make use of in the same way as the allopathists did formerly — i.e., in gastralgias and the like. In these cases of internal application the antiseptic effect is, however, doubtful save in the case when the intestinal flora is concerned.

So far the cannabis preparations — hashish preparations — have been frequently investigated therapeutically, particularly in neurology and in psychiatry, but they were abandoned because the results achieved were not uniform. Nevertheless it would be advantageous to utilize the analgetically sedative effect without the narcotic action of the hashish. Burroughs Wellcome & Co. manufacture a special product: Cannabine Tannate — cannabis combined with tan, *Hydrastis canadensis* and *Secale cornutum* — which has a sedative effect in metrorrhagias and in dysmenorrhagias. From earlier times, otorhinolaryngologists have preserved a prescription against *tinnitus aurium:* ZnO, Extr. *Valerianae*, Extr. *Hyoscyami*, Extr. *Cannabis* āā 1.6 M.f. pill No. 60, D.S. 3-5 pills daily per os. Finally, some years ago, in the *American Journal of Pharmacy*, vol. II (cit. Dinand (18)), the following treatment was recommended in migraine: before meals take for a fortnight conscientiously and daily 0.015 g Extr. *Cannabis indicae*, the third week 0.02 g, the fourth week 0.03 g, to be continued for some months. In all these cases it is the action on the central nervous system which makes its influence felt. Of rather particular interest is the frequent combination of cannabis with tan

FIGURE 2
Cannabis sativa — *engraving from the Czech edition of Mathioli's herbal (63) from the year 1596*

both for internal and external application. We have obtained very good results in stomatitis aphtosa, gingivitis, and in paradentoses with a mouth wash of the following composition: *Tinct. Cannabis* 20.0, *Tinct. Salviae*, *Tinct. Chamomillae*, *Tinct. Gemmarum populi* (or another tan — for example, *Tinct. Gallarum*) āā 10.0, to be applied in the form of sprays or liniments to the inside of the mouth. The use of cannabis as an analgetic but not as an antibiotic in stomatology has been also briefly mentioned by Hegi (34).

In folk medicine particular use is made of the seed. But we also come across the utilization of the shoots for antibiotic and repellent purposes. Around cabbage plants, cannabis plants are grown to repel pieris (caterpillars), and twigs of cannabis are hung in bedrooms to repel gnats and flies.

During the Middle Ages cannabis decoctions were given to cattle for diarrhoea (63, 85). In Argentina cannabis is considered a real panacea for tetanus, melancholia, colic, gastralgia, swelling of the liver, gonorrhoea, sterility, impotency, abortion, tuberculosis of the lungs and asthma. In Argentina (58) even the root-bark has been collected in spring, and employed as a febrifuge, tonic, for treatment of dysentery and gastralgia, either pulverized or in form of decoctions. The root when ground and applied to burns is said to relieve pain. Oil from the seeds has been frequently used even in treatment of cancer;

we have also come across this application in European folk medicine. Also in Argentina, in folk medicine, hemp shoots extracted with butter (*Extr. Cannabis ind. pingue*) are supposed to have a powerful hashish effect, it is believed already, in an amount of 0.1 g; it is employed as a remedy in the Basedow disease. The ethereal extract is less active, and in Argentina it is administered for headache, neuralgia, gout, rheumatism, chorea, melancholia, hysteria, delirium, gastralgia and anorexia. The aqueous macerated product has no narcotic effect at all, and is employed for treatment of tuberculosis of the lungs and as a hypnotic for children and to relieve spastic constipation. An infusion of the leaves is considered to possess a diuretic and a diaphoretic effect. In Europe we also come across many of these uses. Thus Graemer (cit. Dinand (18)) recommends the following for treatment of gastralgia: 0.75 g Extr. *Cannabis ind.*, 10 g ether; 10 drops daily on sugar. For rheumatism a decoction of leaves (15-20 g/0.5 l) is taken internally, and externally poultices prepared of seeds and packings of shreds or tow are used. In Brazil (58) hemp is considered to be a sedative, hypnotic and antiasthmatic remedy. A pronounced antibiotic effect has been observed in South America, where fresh leaves after being ground are used as a poultice for furuncles, and in folk medicine in Europe for treatment of erysipelas (Dinand (18)). Even seed pulp is applied in such cases, but as there are no antibiotics in the seeds we must assume that there is another therapeutic factor involved. In the popular treatment of headache, the plant is preserved in vinegar together with juniper, and the extract is used in form of compresses. Githens (28) and also Watt & Breyer-Brandwijk (89) report on the utilization of cannabis (dagga) in South Africa. There it is smoked because of its narcotic action, but it is also used medicinally. Next to the effect upon the central nervous system we find a considerable use as an antibiotic. For example, the Xosa tribe employs it for treatment of inflammation of the feet. In Southern Rhodesia it is a remedy for anthrax, sepsis, dysentery, malaria and for tropical quinine-malarial haemoglobinuria. The Suto tribe fumigates the parturient woman to relieve pain. These analgetic,[3] sedative and antibiotic properties of cannabis in internal and external application are well known to African tribes.

It may be concluded that ancient and folk medicine have utilized cannabis as an antibiotic and analgesic externally and later as a sedative internally. Narcotic effect has been observed in hot climates only.

2. A brief survey of the methods of isolation and the physical and chemical properties and structures of the isolated antibacterial substances

By F. Santavý & Z. Krejcí

During the last two decades, attention has been drawn to the isolation and toxicology (1, 11, 42, 45) of the substances contained in the plant, *Cannabis sativa*. The following substances have been isolated: cannabinol (5, 12, 15, 93, 94), cannabidiol (5, 12, 38), tetrahydrocannabinol (92), quebrachitol (*l*-inositolmonomethylether) (5), *p*-cymen (79), 1-methyl-4-isopropyl benzen (79), the so-called cannabol (37) of phenolic character which yields a readily crystallizing ester with the chloride of the azobenzencarbonic acid and an optically active and volatile material which was not studied in more detail. In addition, from the individual parts of the plant a series of less important substances has been isolated and listed in detail in Wehmer's compendium (90). The quantitative changes of carotene found present in *Cannabis sativa* have been investigated by Lebedev (54).

Moreover there exists a series of communications (10, 15, 26b, 31b, 57b, 65b) concerned with the isolation and study of substances contained in cannabis which can be extracted from the petrol extract due to its solubility in alkaline lye or in sodium carbonate. The most detailed work in this line was carried out by Todd *et al.* (57b). But none of the authors succeeded in isolating from that portion any acid in crystalline form,—i.e., either an acid itself or its derivative—designating it or determining its constitution. Probably it was the amorphous substance isolated by us and the cannabidiolic acid which was studied further that Todd *et al.* had in hand (see below) but they conclude: ". . . it is concluded that the alkali-soluble portion of the resin contains esters of cannabidiol and cannabinol with a phenolic acid ".

Among the isolated and identified substances mentioned above, cannabinol, cannabidiol and tetrahydrocannabinol proved to be pharmacologically interesting with regard to the hashish effect; a specific hashish-effect is produced only by tetrahydrocannabinol.

All these compounds have already been prepared synthetically and, in addition, a series of homologous derivatives has been prepared for the purpose of establishing the relationships between the constitution and the biological effectiveness (1, 11, 42, 45).

At this stage of the development of the investigation of substances contained in *Cannabis sativa* we began a new line of investigation when one of us (46, 47) found that there are substances contained in it which show an antibiotic effect upon some micro-organisms.

(a) *Starting material*

Fresh or dried pistillate flowers of the cannabis plant (*Cannabis sativa var. indica*) (fig. 3) were used in order to extract the active substances. A plant was used which was not cultivated in a tropical climate, but in the temperate climate of Czechoslovakia for more than five years. We know perfectly well that this factor had undoubtedly a considerable influence both upon the quantitative production of the effective substances or, respectively, upon the composition of the substances contained in the cannabis, and this has been also confirmed by Pulewka (71). Consequently, it may be assumed that this inconstant " variety indica " has become closely similar to *Cannabis sativa*, a plant grown for industrial purposes. Positive results have also been obtained when investigating effective substances contained in *Cannabis sativa* L., a plant commonly cultivated particularly in Slovakia for the industrial utilization of the hemp fibre. We succeeded in showing that the drug from this plant contains antibacterially effective

[3] It is perhaps also of interest that cannabis greatly increases the hypnotic effect of barbiturates.

A B

FIGURE 3

Cannabis sativa var. indica — *plant shoots*

A — Flowering shoots of the pistillate plant (rich in resins).
B — Flowering shoots of the staminate plant.

substances. Unfortunately, the drug from the warmer, tropical or subtropical regions was not available for comparison. The drug obtained from Romania and Yugoslavia did not vary fundamentally in comparison with the raw material available in Slovakia. It must be born in mind that the various cannabis species cultivated in the same climate differ considerably with regard to the quantity of the biologically active substances present; the quantity of the substance amounted from about 0.2 g to 25 g/kg. Likewise, the varying quality of the period of vegetation (dry, rainy, warm and cool weather and also the manure (60, 61)) produces a considerable effect upon the production of the resin and, subsequently, of the biologically active substances. The best, and most valuable raw material yielding antibacterial substances and containing on the average 1-1.5% effective resin substances has been found at the stage when the seed is mature.

(b) *Method of isolation*

The preliminary experiments showed that the biologically effective substances (46-50) of the resin from *Cannabis sativa* may be easily extracted with ether; the paper chromatography revealed that highly polar substances were being dealt with.

After having carried out a series of preliminary experiments, our method of isolation was as follows.

Fresh and rapidly dried tops and leaves of *Cannabis sativa* were extracted in the cold in a percolator with benzene or, preferably, with petrol ether. After partial distillation of the solvents under reduced pressure at 30°C, the residue was carefully extracted with 4% natrium hydroxide, into which both the acids and the substances of phenolic character could be taken up. They were extracted with chloroform after acidification.

First of all the oily residue was shaken in a nitrogen atmosphere[4] with an aqueous $NaHCO_3$ solution with which the acids, particularly the cannabidiolic acid, were extracted to yield a slightly yellow product. By shaking with $NaHCO_3$ an oily residue was obtained which was shown to consist in the main of substances of phenolic character. Both the acid and the phenolic fractions produced an antibacterial effect upon some micro-organisms.

TABLE 1

Output and biological activity of the single fractions isolated from cannabis

	Output [a] (grammes)	Biological activity
Petrolether fraction	59.0	+++
Ether fraction	0.6	+
Chloroform fraction	0.2	—
Ethanol-chloroform fraction	0.05	—
Aqueous residue	6.3	—
Phenolic constituent		+++
Acetylated phenolic constituent		++
Acid constituent (cannabidiolic acid)		++++
Acetylated cannabidiolic acid		+++
Acetylated and hydrogenated cannabidiolic acid		++++
Cannabidiol		+++
Trans-cinnamic acid		—

[a] Values referred to 1 kg of dried material.

After three reprecipitations the acid fraction was allowed to dry over a long period under water pump vacuum at 20°C to yield a colourless glassy substance, the empirical formula of which is $C_{22}H_{30}O_4$; $[\alpha]_D^{24}$ −95° ± 8° (c = 1.00 in chloroform); $[\alpha]_D^{24}$ −115° ± 4° (c = 1.05 in ethanol). The substance crystallized readily after acetylation, m. p. 80-100°/127-128°,[5] $[\alpha]_D$ −114° ± 4° (c = 1 in ethanol).

Repeated isolations showed that the antibiotically effective cannabidiolic acid from *Cannabis sativa* is very sensitive to atmospheric oxygen (particularly in a warm atmosphere) and, furthermore, that the acid undergoes changes due to higher temperature and a simultaneously reduced pressure.

[4] Modification according to Schultz & Haffner (77).

[5] From some of the crops the acetylester of the acid, m.p. 95-100/110-115° was obtained. According to our assumption, there are either two isomers present whose occurrence is conditioned by the climate, or these substances are interconvertible.

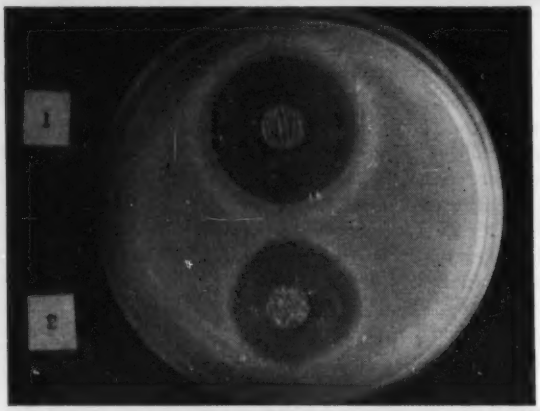


FIGURE 4

Rate of inhibition of the acid and phenolic fraction of the extract from cannabis upon the inoculated staphylococcus (meat peptone broth)

1 — Acetylated fraction from which the cannabidiol acid crystallized.
2 — The acetylated phenolic residue.

(c) *Determination of the structure*

As previously mentioned, the biologically active acid isolated from *Cannabis sativa* has the empirical formula $C_{22}H_{30}O_4$, and yields, when acetylated, a diacetyl derivative $C_{26}H_{34}O_6$ whose extinction curve in the UV-range closely resembles that of the cannabidiol derivatives. At the beginning of our experimental work, therefore, the acid obtained by us was already called cannabidiolic acid (49, 59).

By boiling with alkali, the acetylated cannabidiolic acid is saponified and partially decarboxylated, which results in precipitation of a substance of phenolic character from the solution, particularly after its saturation with carbon dioxide. That the substance undergoes decarboxylation was best seen when we tried to purify the proper acid isolated by us or the acid liberated from its acetylester by the help of sublimation. The process gives rise to cannabidiol, a substance characterized by the positive Beam-reaction and as 3,5-dinitrobenzoate. This supports our former view (49) that, regarding the structure, the antibacterially effective acid, isolated from *Cannabis sativa*, is closely related to cannabidiol.

The position of the carboxyl group in the molecule of the cannabidiolic acid was studied along the physical-chemical pathway (51, 52).

On comparing the optical rotation of the cannabidiolic acid, its acetyl derivative and its tetrahydro derivative with the cannabidiol (2, 3) derivatives we found a remarkable correspondence. An analogous agreement exists (4) between the derivatives of the tetrahydrocannabinol and hexahydrocannabinol; acetylation and methylation do not bring about a significant change in the degree of the optical rotation. The percentage rate of the molecular optical rotation of the hydrogenated and the unhydrogenated derivatives is, as far as these three groups are concerned, practically the same. The optical rotation changes only with the varying position of the double bond of the B-ring and because of the formation

TABLE 2

Values of the optical rotation of the single substances (in ethanol)

(Degrees)

Substance	$[L]_D$	D
Cannabidiolic acid and its derivatives		
Cannabidiolic acid	—115	—412
Diacetyl derivative	—114	—504
Tetrahydrodiacetyl derivative	—22	—98
Cannabidiol and its derivatives		
Cannabidiol (2)	—125	—393
3,5-dinitrobenzoate (2)	—76 *	—513
Dimethylether (2)	—133	—455
Monomethylether (2)	—118	—373
Tetrahydrodimethylether (3)	—28	—89
Tetrahydrodiacetyl derivative (3)	—30	—104
Tetrahydrocannabinol and its derivatives (1 b, 4)		
Isomer I	—267	—834
Isomer II	—130	—406
Hexahydrocannabinol	—70	—220

* Acetone

of an ethereal bridge due to conversion of cannabidiol into tetrahydrocannabinol. From the above it may be concluded that the carboxyl group of the cannabidiolic acid does not seem to be attached to some carbon of the isocyclic nucleus where it could lead to the formation of a further optically active centre, but that it is attached either to the aromatic nucleus or to some other carbon where an optically active centre cannot be formed. The ready decarboxylation excludes its position on the carbons 7, 9 and 15.

In addition, we have tried to determine the position of the carboxyl spectroscopically.

The acetyl derivative of the cannabidiolic acid shows an inflexion in the UV-range, or a maximum at the same wavelength as cannabidiol (94), or its derivatives (fig. 5). Analogously, the same maxima are produced by the diacetylester of the hydrogenated cannabidiolic acid.

Evidently, therefore, the carboxyl group of the cannabidiolic acid is not conjugated with the double bond of the nucleus B.

Still more striking is the behaviour of the carboxyl group

TABLE 3

IR-frequency values within the carbonyl range

	Concentrated solution	Diluted solution	Dioxan
	In chloroform		
Acetylester of the cannabidiolic acid	1698 cm^{-1}	1700 cm^{-1}	1728 cm^{-1}
Hydrogenated acetylester of the cannabidiolic acid	1698 cm^{-1}	1700 cm^{-1}	1728 cm^{-1}

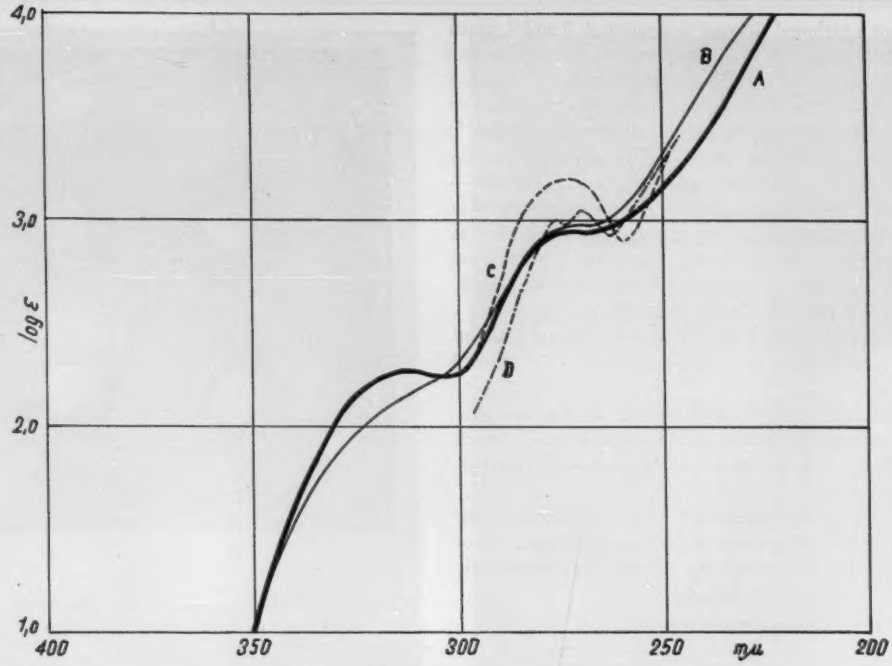


FIGURE 5

UV-spectra of the cannabidiol derivatives (in ethanol).

A — Acetylester of the cannabidiolic acid. B — Hydrogenated acetylester of the cannabidiolic acid. For comparison the curves of the dimethylether of the cannabidiol (C) and of the dimethylether of the dihydrocannabidiol (D) have been taken from the literature (6).

in IR-spectroscopy (fig. 6).[6] We attribute the frequency at 1770 cm^{-1} to both phenoacetoxy groups. The frequency at 1698 cm_{-1} to the free carboxy group, or to its carbonyl, respectively. This frequency does not vary, either with the acetylester of the cannabidiolic acid or with its hydrogenated product (cf. table 3).

With regard to the fact that the frequencies of the carbonyl of both acetoxy compounds (i.e., of the saturated and of the unsaturated) are found to be the same, it may be assumed

[6] Our thanks are due to Dr. Horák, Institute of Chemistry, Academy of Sciences, Prague, for the measuring of the infra-red spectra and their evaluation.

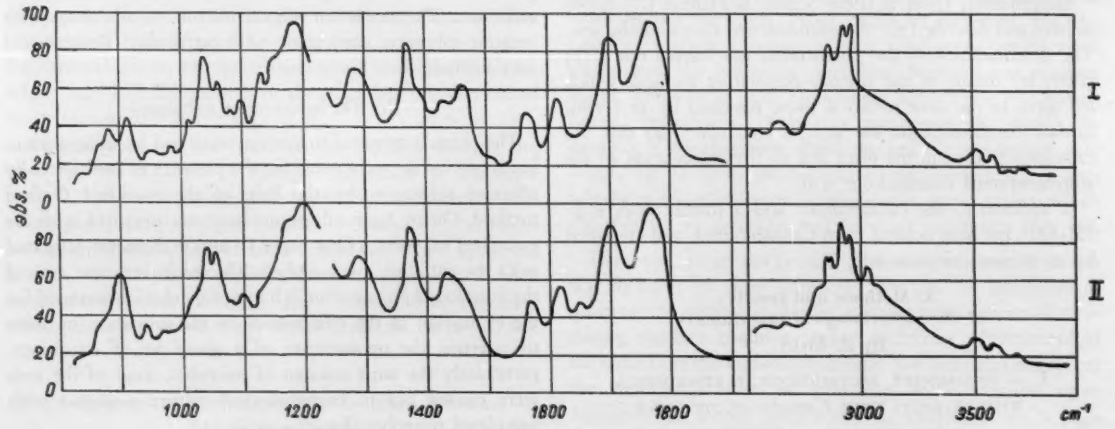


FIGURE 6

IR-spectra

I — Acetylester of the hydrogenated cannabidiolic acid.

II — Acetylester of the cannabidiolic acid. Both compounds have been solved in chloroform.

that it is not a carboxyl situated in position 4, 5 and 9 which is being dealt with, for the disrupture of the conjugation due to reduction would be followed by a shift of the frequency to higher values. On the contrary, the frequencies observed in chloroform (1698 cm^{-1}) and in dioxane (1728 cm^{-1}) evidence again that it is not the alicyclic acid (i.e., in position 1,2,3,6) — namely, one whose carbonyl is not conjugated — for the frequencies of such acids have been found to appear in the higher frequency region (in chloroform at about 1715, in dioxane at about 1740 cm^{-1}). But there was a very good agreement to be seen with regard to the frequency of benzoic acid (in chloroform 1694, in dioxane 1724 cm^{-1}).

If we take it for granted that there is a carboxyl attached to the aromatic nucleus, it is only conceivable in position 3 (or 5, respectively).

The frequency at 1615 cm^{-1} belongs to the aromatic vibrations and the frequencies at 900 and 1650 cm^{-1} which disappear during hydrogenation must be attributed to methylene in isopropylene.

On the basis of the organically preparatory experiments carried out so far, comparison of the optical rotation and of the UV- and the IR-spectra, the formula for cannabidiolic acid suggested by us is as follows:

(3-methyl-6-isopropenyl-4'-n-pentyl-2',6'-dihydroxy-1,2,3,6-tetrahydrodiphenyl-3'-carboxylic acid)

The arrangement of the substituents of the nucleus A of the cannabidiolic acid, as formulated by us, does not appear to be exceptional among the natural substances obtained from plants. If, in the case of the cannabidiolic acid, we do not take into consideration the partially aromatic nucleus B, we obtain the olivetolic acid (7) which has been found present in numerous plants (8). This substance is also readily decarboxylized and thus converted into olivetol which forms the aromatic moiety of cannabidiol. There are also some deeply related reactions ($FeCl_3$, $CHCl_3$ + 10% KOH, Vanillin + HCl) to be found in both groups of these substances.

Independently from us (1955) Schulz & Haffner (77) (1958) isolated and described the above-mentioned cannabidiolic acic. The determination of the constitution was carried out (77b) (1959) by means of the organic-preparative methods which led them to the same results as those obtained by us (1958). In their communication the authors conclude (77c) that the cannabinols occur in the plant due to decarboxylation of the aforementioned cannabidiolic acid.

In addition to the cannabidiolic acid a further acid, m.p. 131-133°, has been isolated from *Cannabis sativa*, and identified by us as *trans*-cinnamic acid.

3. Methods and results of the baeteriologieal experiments

By Z. Krejcí

I. — Preliminary bacteriological experiments with extracts from *Cannabis sat. var. indica*

(a) *Preparation of extracts for the preliminary microbiological evaluation*

It was clear from the beginning of our experimental work that the antibiotic effect of the drug must stand in close relationship with the content of the resin substances contained in the leaves and, particularly, in the shoots of the pistillate plant (fig. 3). The ground drug was extracted by the method devised by Carlson (13, 14). The different parts of the plant (roots, stems, leaves, shoots and seeds) were extracted at higher temperature, normal room temperature and even at 1°C, at the ratio of 1: 5, with ethylalcohol, ethylether, a physiological solution, 1.5% sulphuric acid, and 1% sodium hydrocarbonate. The extraction was carried out to advantage with organic solutions, particularly with petrolether, benzene and ethylalcohol.

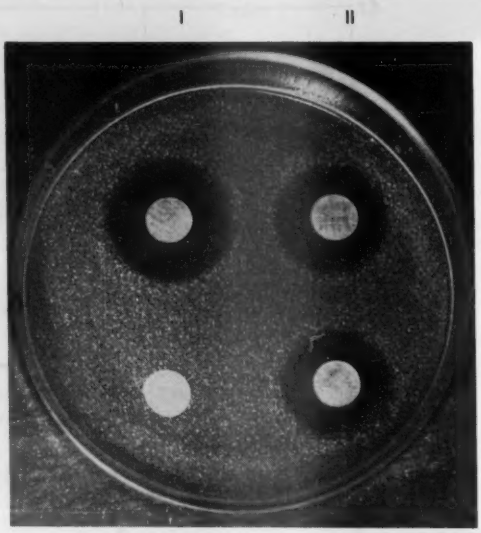


FIGURE 7

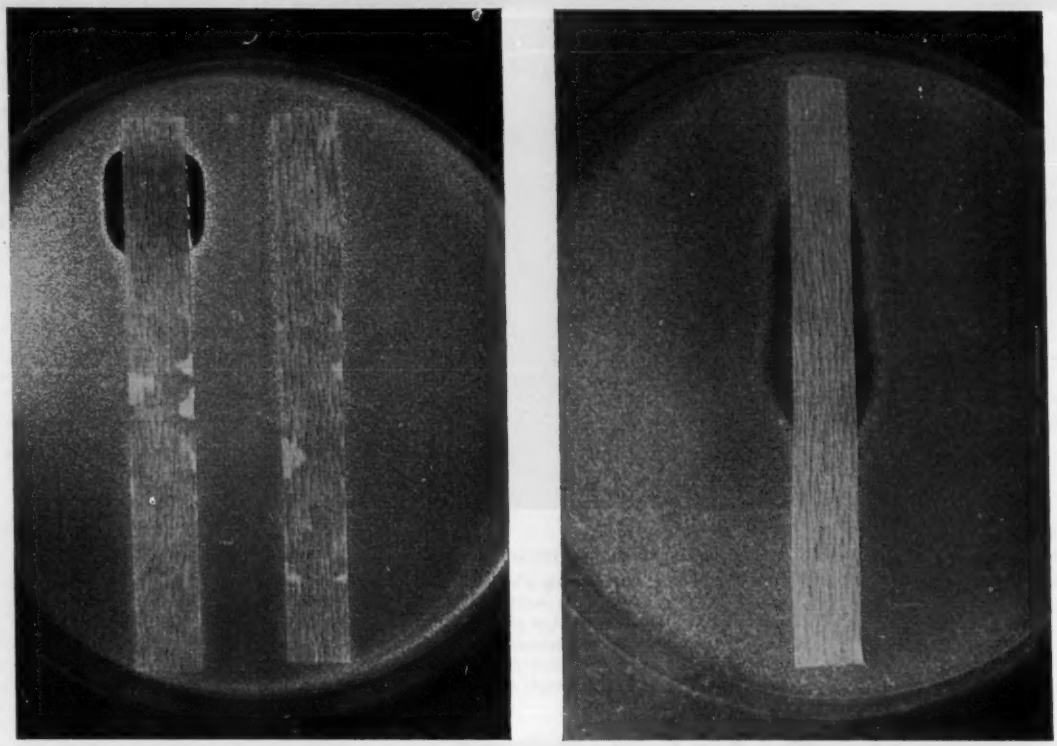
Rate of the inhibitory effect of the raw extract from cannabis upon Staphylococcus aureus *(modified Oxford method). One drop of the extract has been used for carrying out the test.*

I — *Extract in 70 per cent ethyl alcohol.*
II — *Extract in 96 per cent ethyl alcohol.*
III — *Diethyl ether extract.*
IV — *Control test with 96 per cent ethyl alcohol.*

(b) *The bacteriological technique*

The extracts prepared in various ways and by using various kinds of solvents were tested for the presence of antibacterially effective substances by the help of the modified Oxford method. On an Agar culture medium contaminated with the examined microbe, a filter paper 10 mm in diameter, saturated with the antibiotic, was applied. The inhibition zone around the butts found present after 24 hours of incubation was used for the evaluation of the effectiveness of the substance. In order to ascertain the maintenance of a given set of conditions, particularly the same amount of microbes, most of the tests were carried out in bacteriological culture mediums with inoculated microbes (fig. 7).

The other and more detailed investigations (the rapidity of the effect, the inhibition factors and so on) were carried out routinely in the fluid medium of peptone water or, with the aid of Sauton culture mediums (BK), respectively.



a b

FIGURE 8

Chromatography illustrating the inhibitory effect upon the growth of the staphylococcus around the antibiotically active zone; undimensional ascending paper chromatography. b *shows two zones running into each other (cf. details in the text).*

The preliminary experiments concerning the isolation of biologically active substances were performed by the help of the ascending chromatography or the capillary analysis. The antibacterially effective fractions were detected by placing the chromatograms into inoculated culture mediums. The zone which inhibited the growth of the microbes was considered to be biologically active (fig. 8a, b).

An analogous method based on the capillary analysis and used for the isolation of effective substances is illustrated in fig. 9. The terminally situated zone in the ring chromatogram when placed into an Agar medium inoculated by staphylococcus produces a considerable inhibitory effect upon the growth of the above-mentioned micro-organisms. Further work showed that the chlorophyll contained in the plant extract does not produce any direct antibiotic effect (66, 84).

(c) *Spectrum of bacteria sensitive to active substances obtained from cannabis*

The investigations were carried out with specimens of gram-positive micro-organisms — i.e., *Staphylococcus pyogenes aureus* — and from the gram-negative series the *Escheria coli* were used. The extracts produced a remarkable bactericide effect upon *Staphylococcus aureus*, whilst *E. coli* showed to be resistant. On the basis of these preliminary findings we have aimed our work in this direction. Proof could be furnished that the cannabis extracts produce a very satisfactory antibacterial effect upon the following microbes: *Staphylococcus pyogenes aureus, Streptococcus alpha haemolyticus, Streptococcus beta haemolyticus, Enterococcus, Diplococcus pneumoniae, B. subtilis, B. anthracis, Corynebacterium diphtheriae* and *Corynebacterium cutis* — i.e., all of them gram-positive micro-organism. Noteworthy is the effect upon *Staphylococcus aureus* strains, which are resistant to penicillin and to other antibiotics (fig. 10).

That was one of the peculiar properties of cannabis which was found to be most attractive. We saw the possibility of utilizing the antibiotic locally without any danger of producing resistant strains to other antibiotics administered at the same time throughout treatment. We must also remember the very good effect of substances from cannabis upon *Staphylococcus aureus*, particularly nowadays, when a high percentage of staphylococcus diseases offers resistance to penicillin. It is interesting that staphylococci manifesting various degrees of resistance to one or more antibiotics (erythromycin included) are sensitive to the antibiotics from cannabis in the same degree throughout. So far, we have not

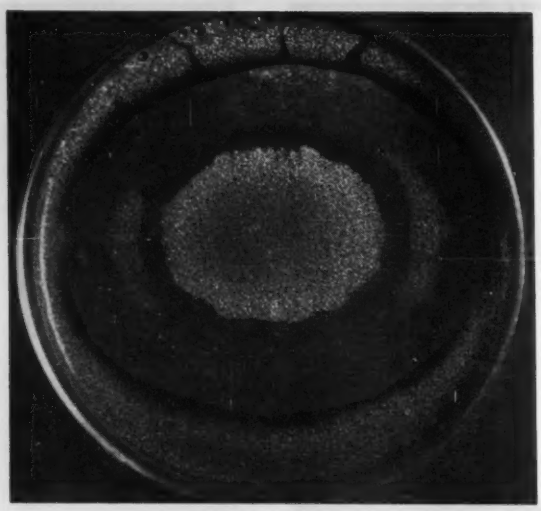


FIGURE 9

Inhibitory effect upon staphylococcus when placing the circular paper chromatogram — capillary analysis — into inoculated Agar. The light centre and the spots around the periphery of the Agar plate indicate the boundary of the inhibitory zone produced by the active substance. It appears that there are two biologically active substances present whose partition factors show to be different; evidence in this respect could be brought forward chemically.

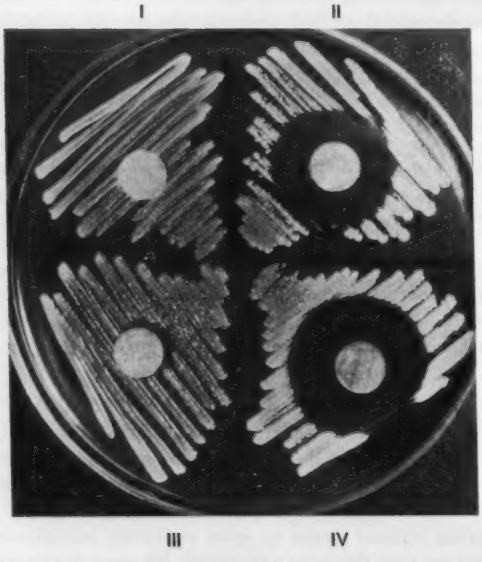


FIGURE 10

A parallel drawn between the rate of the inhibitory effect of the extract from Cannabis indica *and of penicillin upon penicillin resistant strains of* Staphylococcus aureus

I — *2% acidum carbolicum liq.*
II — *1,000 OU of penicillin.*
III — *Hemp extract in 70% ethyl alcohol.*
IV — *Hemp extract in 96% ethyl alcohol.*

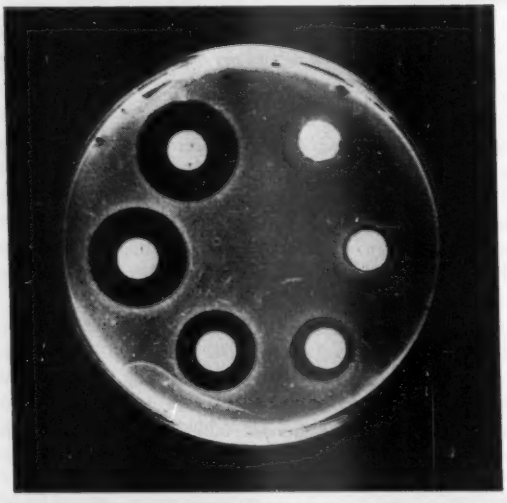


FIGURE 11

Preliminary titration of the IRC-effect upon Staphylococcus aureus *(Oxford method). Concentrations: 2%, 1%, ½%, 1 : 1,000, 1 : 10,000, 1 : 100,000*

observed that any resistance of the staphylococcus strains to these substances would arise. The degree of the artificially produced resistance to these substances, as shown by Martinec (62), corresponds with the origin and the degree of resistance to substances of the phenolic type and to other disinfectants.

The tests carried out with other micro-organisms, particularly with gram-negative strains, just as well as with fungi and yeast, gave negative results (table 4). On the contrary our work evidenced the positive effect of the isolated substances upon *Mycobacterium tuberculosis.*

TABLE 4

Effect of the Cannabis indica resin upon some common pathogene micro-organisms

Investigated strain	Effect
1. Micrococcus albus	Positive
2. Staphylococcus pyogen. aureus haemolyt. sensitive to penicillin	Positive
3. Staphylococcus pyogen. aureus haemolyt. resistant to penicillin	Positive
4. Streptococcus alpha haemolyt.	Positive
5. Streptococcus beta haemolyt.	Positive
6. Enterococcus	Positive
7. Diplococcus pneumoniae	Positive
8. Erysipelothrix rhusiopath.	Positive
9. Sarcina lutea	Positive
10. Corynebact. diphtheriae	Positive
11. Corynebact. cutis	Positive
12. Bac. anthracis	Positive
13. Bac. subtilis	Positive
14. Bac. mesentericus	Positive
15. Clostridium perfringens	Positive
16. Escherichia coli	Negative
17. Salmonella typhi	Negative
18. S. paratyphi B	Negative
19. Sh. Shigae (Sh. Flexneri, Sh. Kruse Sonnei)	Negative
20. Pseudomonas aeruginosa	Negative
21. Proteus vulgaris	Negative
22. Mycobacterium tuberculosis	Positive

II. — BACTERIOLOGICAL EXPERIMENTS CARRIED OUT WITH ISOLATED AND PURIFIED RESIN FROM CANNABIS

As previously mentioned in the section on chemistry, both the perfect extraction of the effective substance and the method of isolation of the resins have been elaborated and patented (53). Whilst the preliminary investigations were carried out with hemp extracts obtained by the help of some organic solvents (ethylalcohol, ethylether, petrolether, benzene), the proper work and study of the antibacterial effect were accomplished throughout by the help of the purified substance of resin nature, further on designated as the IRC (Isolated Resin from Cannabis), from the viewpoint of chemistry a mixture of acid and phenolic fractions; hence, we have used not the crystalline forms which have been shown to be less effective but the mixture mentioned above — the resin. The crystalline forms have been found particularly useful for the evaluation of the chemical structure, the determination of the chemical and physical properties and the constitution

TABLE 5

Velocity of the bactericide effect of various IRC-concentrations upon the investigated micro-organisms

IRC-concentration	Velocity of the effect when adding antibiotics
1 : 100	Immediately sterile
1 : 1,000	Sterile after 15 to 30 minutes
1 : 10,000	Sterile after 3 hours
1 : 100,000	Sterile after 8 hours
1 : 1,000,000	After 24 hours remarkably inhibited growth
Control without IRC	Massive growth and opacity

of the effective substances. Consequently, all the results mentioned below will refer to the IRC, tested upon *Staphylococcus aureus haemolyticus.*

(a) *Determination of the magnitude of the bactericide effect*

It could be demonstrated by us that the IRS produces a pronounced bactericide effect *in vitro*; in lower concentrations

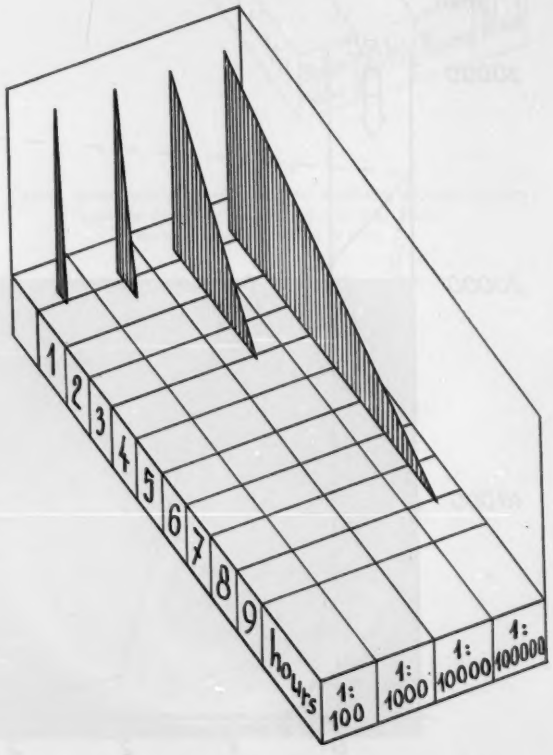


FIGURE 12

A tridimensional graphic representation of the velocity of the bactericide effect of various IRC-concentrations upon staphylococcus aureus *in fluid media* in vitro

the growth and reproduction of sensitive bacteria are but slowed down. The rate of the effect in vitro has been tested both by the help of the modified Oxford method on solid culture mediums and in fluid mediums of peptone water. The Oxford method was shown to be by far the least sensitive. There were shown to be many factors involved which have no connexion with the antibiosis as, for example, diffusion, the number of inoculated microbes and so forth. It can be utilized with advantage only for purposes of orientation (fig. 11). Whilst the maximum of the effect of the IRC-dilution was found to be with a concentration of about 1: 10,000 when the Oxford method was used, the tests carried out in the fluid medium of peptone water evidenced a bactericide effect still in a dilution of 1: 100,000 after inoculation within 24 hours, and a significant inhibition of the growth in a dilution of 1: 1,000,000.

The antibacterial magnitude of the IRC-effect in ethylenglycol upon the BK H/37 RV strain in a Sauton fluid medium was found by titration up to a dilution of 1: 100,000. In detailed work carried out in the Laboratory for Bacteriology of the Sanatorium at Vyšné Hágy (High Tatra) where independent tests for control were carried out, the limit of the effect upon the same strain of Mycobacterium tuberculosis was determined to be present up to a dilution of 1: 150,000.

TABLE 6

Velocity of the bactericide IRC-effect upon Staphylococcus aureus

Time control	IRC 1 : 100	IRC 1 : 1,000	IRC 1 : 10,000	IRC 1 : 100,000	Control
	Number of colonies				
Immediately after application of antibiotics..	—	5	8,640	20,600	25,000
After 15 minutes	—	3	560	20,500	
After 30 minutes	—	—	37	16,000	
After 45 minutes	—	—	—	20,300	Proportional increase of the number of the micro-organisms
After 1 hour	—	—	29	18,800	
After 2 hours	—	—	12	1,760	
After 3 hours	—	—	14	278	
After 4 hours	—	—	—	240	
After 5 hours	—	—	—	32	
After 6 hours	—	—	—	21	
After 8 hours	—	—	—	4	
After 12 hours	—	—	—	—	
After 24 hours	—	—	—	—	Massive growth

(b) *Determination of the velocity of the bactericide effect of the various IRC-concentrations upon tested strains*

The velocity of the bactericide effect was determined with the aid of the routinely used method in the medium of pep-

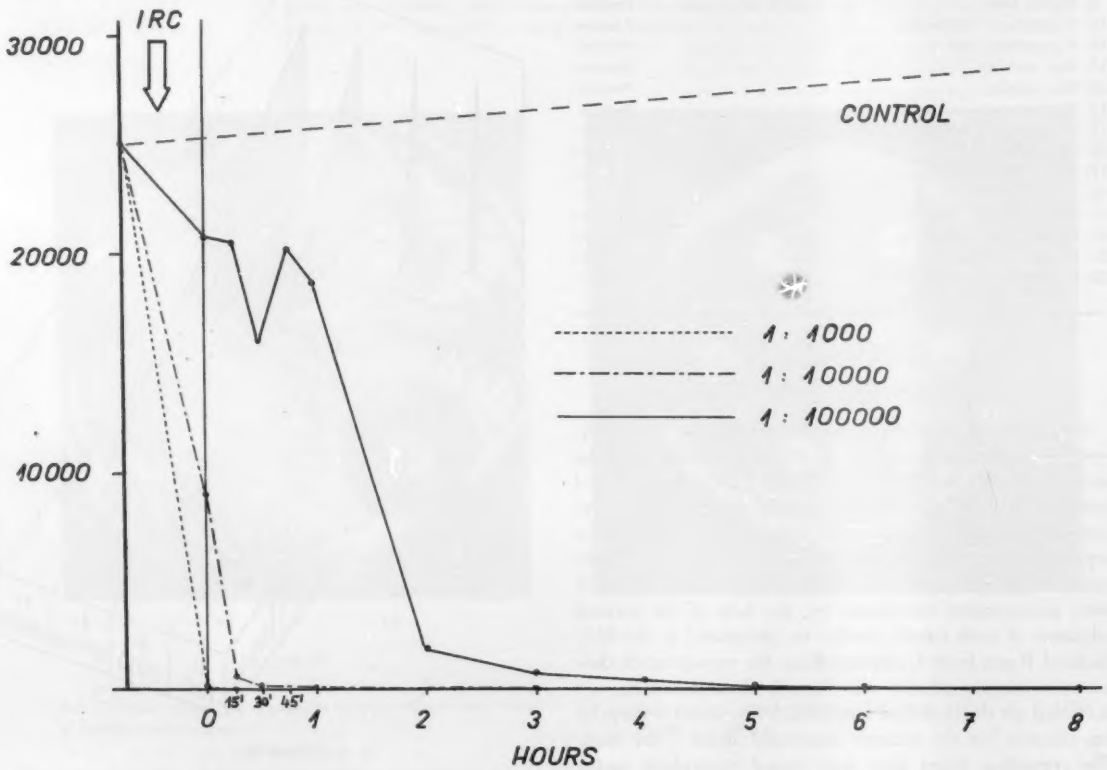


FIGURE 13

Graphic representation of the decrease of the number of micro-organisms after various time-intervals and in different IRC-concentrations

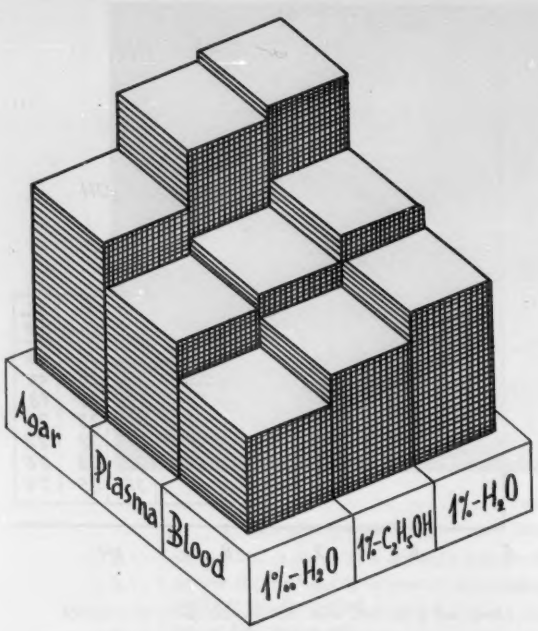


FIGURE 14

Graphic representation of the influence of the inactivating substances in vitro *upon the decrease of the IRC-effect in solid culture media*

Resin isolated from *Cannabis indica*	Agar	Agar-plasma	Blood-Agar
1‰ aqueous solution	20	15	11
1% alcohol solution	26	16	14
1% aqueous solution	27	19	17

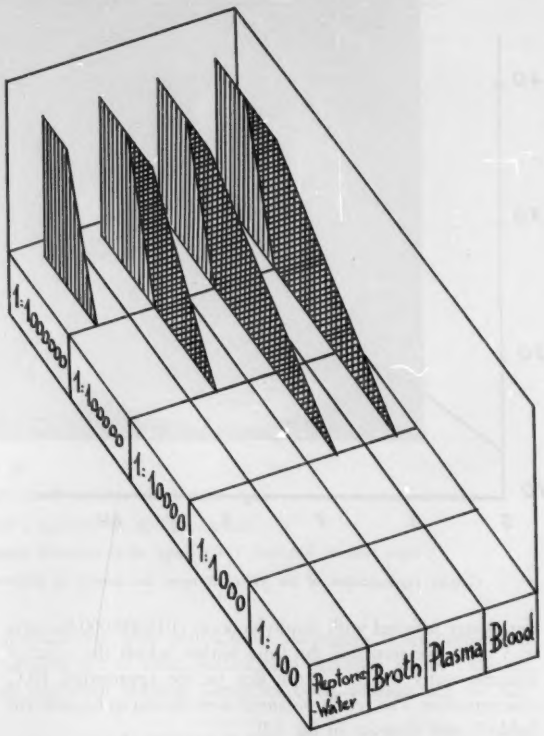
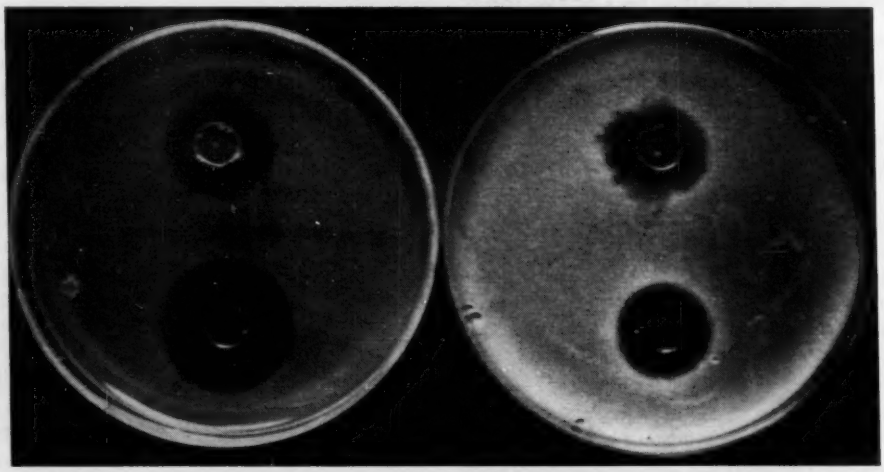


FIGURE 16

Graphic representation of the influence of the inactivating substances in vitro *upon the decrease of the IRC-effect in fluid media; evaluated after a period of 24 hours*



1 FIGURE 15 2

Effect of the inactivating substances in vitro *upon the decrease of the antibacterial IRC-effect in:*

1 — Meat-peptone Agar.

2 — Agar with 10% plasma.

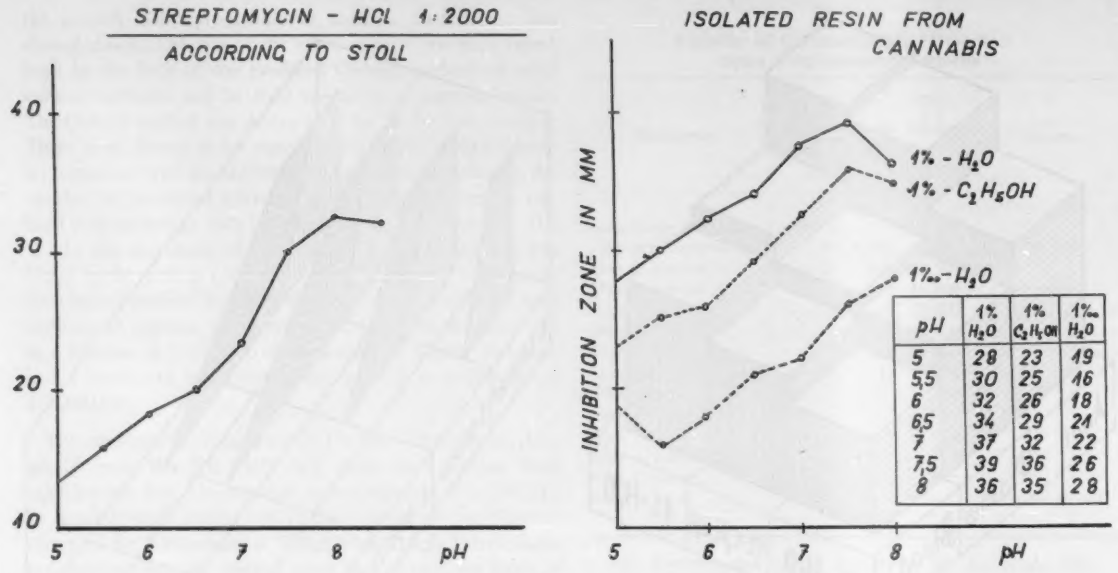


FIGURE 17

Graphic representation of the pH-effect upon the activity of streptomycin (Stoll) and of the IRC when staphylococcus species are concerned

tone water infected with staphylococcus (100,000,000 bacteria in 5 ml). We recorded the time within which the infected peptone water became sterile due to the appropriate IRC concentration. The results obtained were shown to be uniform (table 5, and diagram in fig. 12).

Analogously we followed the decreasing number of bacteria. Fifty cc of sterile peptone water were infected with a diluted 18-hour-old *Staphylococcus aureus* culture which, according to the control, contained 30,000 micro-organisms in 1 cc. Into each of the individual flasks the IRC was added, progressively diluted from 1: 100 up to 1: 100,000 and, with the aid of the usual method of cultivation, and after definite time intervals, the decreasing number of bacteria in 1 cc was recorded (table 6, and diagram in fig. 13).

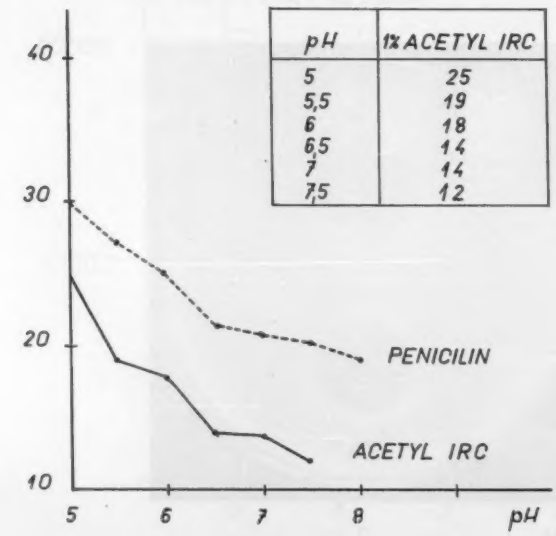


FIGURE 18

Graphic representation of the pH-effect upon the activity of penicillin (Stoll) and upon the acetyl derivative of the acid from cannabis

(c) *Substances inactivating the antibacterial effect of the IRC*

A detailed study has been carried out *in vitro* on solid and in fluid culture mediums with regard to the effect of blood, serum, cysteine, thiosulfate, meat peptone broth and Sauton medium upon the bactericide magnitude of the IRC. All the substances mentioned above, save for cysteine and thiosulfate, showed a decrease in the biological effect to a larger or smaller extent (fig. 14, 15). Whilst in the standard peptone water there could be observed a bactericide effect upon the staphylococcus up to a concentration of 1: 100,000, under the same conditions after addition of an amount of 10 per cent blood or plasma the concentration showed itself active only up to a dilution of 1: 1,000. It is interesting to notice that bloods of various origin, and even of different groups, do not produce the same inactivating effect. In the same way, in comparison with the medium of peptone water, the peptone broth showed a decrease in the bactericide effect by one degree (fig. 16).

(d) *Effect of the pH-value upon the antibacterial effect of the IRC*

Stoll et al (83) published a report dealing with the problem of the dependence of the magnitude of the antibacterial effect upon the pH-value with regard to penicillin and streptomycin. They found that at lower pH-values the anti-

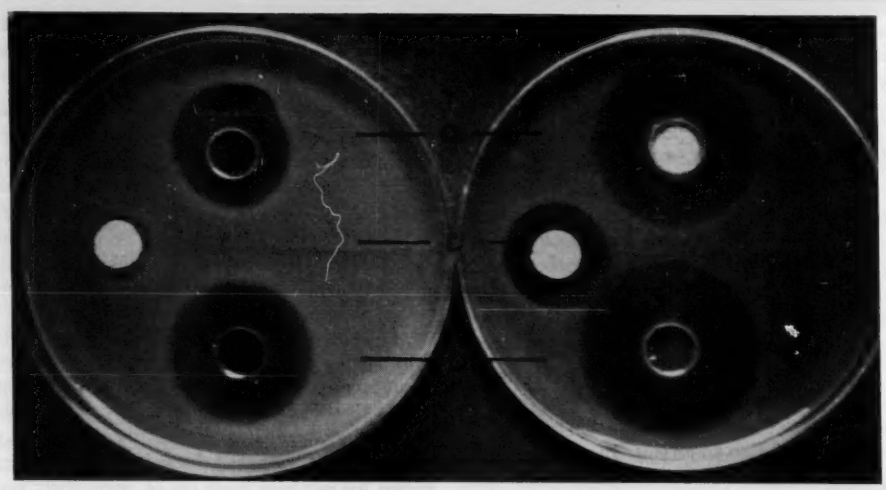


FIGURE 19

pH-effect upon the IRC-action upon Staphylococcus aureus *in meat-peptone Agar*

a — *1 per cent IRC (sodium-salt) in alcohol (5 drops placed into a depression in the Agar-surface).*

b — *1 per cent IRC (sodium-salt) in water (5 drops placed into a depression in the Agar-surface). (one drop on filter paper applied to the Agar-surface).*

c — *1 per cent IRC (sodium-salt).*

biotics of acid character produce a more pronounced effect than at the pH-value within the alkaline range (penicillin). On the contrary, the effectiveness of streptomycin rises steeply with the rising pH. In our experiments the same results were obtained. We followed on solid culture mediums, and at pH-values that varied between 5 and 8, the rate of the inhibiting effect upon *Staphylococcus aureus.* The various concentrations tested were as follows: (*a*) natrium salts of the IRC in alkaline aqueous solutions, (*b*) the crystalline acetyl derivative of the isolated cannabidiolic acid.

In the first case the increasing effectiveness of the solution along with the rising pH-values bore analogy to the one of streptomycin (fig. 17).

On the contrary, in the second case, the effectiveness of the acid tested showed an increase on the acid side, which was analogous with the effect of the pH-value upon the antibiotic effect of penicillin (fig. 18).

As may be seen from both diagrams, there is a noticeable difference to be found with regard to the size of the inhibition zones under standard conditions and varying pH. The maximum of sodium salts in water lies at about pH 7.5, subsequently approximately at the pH of the plasma. Less advantageous is the considerable decrease of the effect in an acid medium — for example, of a suppurant wound. The photograph of fig. 19 portrays the considerable disparity as to the effectiveness of the isolated substances at varying pH under conditions which are otherwise maintained. The same concentrations of active substances have been tested in parallel line in an Agar culture medium once at pH 5.5, and the second time at pH 7.5.

On the basis of the dependence mentioned above, conclusions should be drawn as to the practical utilization of the preparations with antibacterial substances from cannabis at varying pH of some pathological processes as, for example, in suppurant wounds, furuncles, otitis, sinusitis and the like.

4. Survey of clinical experiences

By Z. Krejcí

(a) *Some chemical and physical properties of the isolated effective substances (IRC) particularly with regard to their stability*

The antibiotically active and effective substance (IRC) of resinous appearance and nature, utilized at present for the preparation of the antibacterial preparations, does not appear to be a chemical entity, but a mixture of two substances. It is of acid nature conditioned by the presence of phenolic and carboxyl substances. These acid substances retain their antibacterial properties even after acetylation and following division in the neutral portion (acetylated phenols) and the acid one (acetylated-phenol-acid).

Going deeper into the matter showed that the biological effectiveness of the active amorphous substances is not so stable as was previously assumed. On the contrary, their effectiveness decreases due to the oxidizing action of atmospheric oxygen, particularly in a warm atmosphere. In an oxygen-free medium, even at higher temperatures (in the autoclave) they are comparatively stable.

The effective substances are all readily soluble in common organic solvents — namely, in ethylalcohol, ethylether, chloroform, benzene, petrolether and so on. At pH 7 they are slightly soluble in water and in physiological solutions. This property is a certain drawback in these substances both for clinical utilization and precise pharmacological evaluation. Because of their acid nature they are readily soluble in aqueous solu-

tions of alkalis — i.e., in sodium and potassium hydroxide, sodium carbonate, sodium hydrocarbonate and so on. They can be easily emulsified, and as emulsions they are comparatively stable.

(b) *The pharmacodynamic effect of the antibacterial substances from* Cannabis sativa var. indica

The pharmacological evaluation was carried out at the Institute for Pharmacology of the Medical Faculty of the Palacky University. Klabusay & Lenfeld (43) succeeded in showing some well-defined effects of the isolated amorphous substances (IRC): analgetic, anticonvulsant, and, when locally applied, the initially irritative and later on anaesthetic effects.

The *hashish effect* due to tetrahydrocannabinol could not be found in substances obtained from plants cultivated in the moderate climate of Czechoslovakia — i.e., it could be evidenced neither in animal experiments nor in experiments carried out on man (*per os*).

The *analgetic action* was studied in white rats by the help of the method of mechanical irritation according to Levy and Buchel in the modification according to Votava and in guinea pigs by the method according to Regnier. The IRC was administered perorally in emulsions with gum arabic in doses from 100 to 50 mg/kg; and also subcutaneously and intraperitoneally in ethylenglycol up to 500 mg/kg weight. From these experiments it could be seen that the IRC produces an analgetic action when administered perorally in doses of 100 mg, subcutaneously and intraperitoneally — i.e., from 50 mg/kg onward. Higher doses (500 mg/kg) of the IRC result in intensified analgesia lasting for some hours and complete inhibition of the activity of the central nervous system or sleep.

The *anticonvulsive effect* was evaluated in white mice and in frogs. The convulsions were produced in mice by pentamethylentetramine (0.1 g/kg intraperitoneally), in frogs by strychnine by administering doses of 40 mg/kg subcutaneously. The effective dose of the IRC preventing convulsions due to pentazole in 50% of mice was established as 0.61 g/kg *per os*; for strychnine an effective dose of 0.83 g/kg when applied subcutaneously.

The determination of the *local anaesthetic effect* was carried out by evaluation of the surface anaesthesia according to the method of Lebduška & Vrba using the cornea of rabbits and guinea pigs, and by the evaluation of the anaesthesia according to the method of infiltration of Bülbringe & Wayda, using the skin of guinea pigs. The IRC was applied in ethylenglycol. The IRC was effective from the concentration of 5% and up, whereas a total local anäesthetic effect could be observed only by using a 10% concentration; it lasted for about 45 to 90 minutes.

The *toxicity* was determined in mice by administering an emulsion with gum arabic, and the death rate recorded within 48 hours. By the Burn method, LD_{50} was established as 1.83 g/kg *per os*. Smaller doses did not produce any remarkable changes in mice; doses of 1 g/kg (IRC) were followed, after some hours, by a slight inhibition of the motor function with apathy and immobility. Larger doses resulted in a total inhibition of the activity of the central nervous system, sleep and exitus within 24 hours.

The *local compatibility* was evaluated on the cornea of rabbits by administering a solution in drops and by application of the solutions of the tested substances into the subcutaneous area of the auricle of the rabbit. The effect of the IRC upon the mucous membranes was locally irritating. On local application to the skin no clinical evidence of acute irritation was found. The intramuscular application resulted in an inflammatory reaction with the exudation of the liquid.

(c) *Clinical experiences*

In recent years a series of pharmaceutical preparations have been prepared and clinically examined particularly with regard to the needs of stomatology, otorhinolaryngology, gynaecology, dermatology and the like. The preparations have been locally applied throughout but parenteral administration has not been made use of, particularly because of the poor solubility of the examined substances in physiological solutions. Besides, some of the properties such as the pharmacological one (irritation) and the bacteriological one (inactivation with blood and serum) of the active substances which have been isolated do not allow a suitable utilization of these preparations by means of injections.

For the needs of stomatology, an aseptic dentin powder was prepared, impregnated with 2 to 5% of biologically active substances (IRC) from cannabis. This dentin powder containing IRC was successfully used for the indirect covering (in about 300 patients) and for the direct covering of the pulp (70 cases), in beginning pulpitis and irritation of the pulp. Even in cases like that we notice the obvious advantage of the antibacterial effect of the locally anaesthetic action. Save in cases of massively infected pulps, Soldan (81, 82) did not record any failures.

Somewhat less uniform results were obtained with this preparation by Šimek (78), who applied dentin combined with IRC or a special salve containing IRC. In uncomplicated *Caries profunda*, a remarkable and spontaneous effect could be achieved in 64% of the patients; in *Caries profunda*, with an incidental opening of the vital pulp, in 38.4% of the patients. In *Pulpitis partialis*, the results of this therapy were positive in 41% of the investigated cases. The authors have, on the whole, acknowledged the anaesthetic effect of these preparations.

Very advantageous is the application of the effective substances from cannabis in *otorhinolaryngology*. Hubácek (36) reports on the very good results obtained particularly after application of 1% of an alcoholic solution of the IRC and of a dusting powder (boric acid with 2 to 5% of the IRC) in acute and chronic otitis, furunculosis of the outer part of the nostrils and of the external auditory meatus, and even in some cases of bilateral sinusitis, the latter having been treated without success with a series of punctions and penicillin irrigations. He describes an interesting case of bilateral chronic *Sinusitis maxillaris*, lasting for about three years and treated without success with a total of about 30 punctions. After application of our IRC preparations, one side was treated as a control with penicillin, but with negative result, and the

other side with three IRC punctions and irrigations. The side treated with negative effect with penicillin was easily and successfully treated with cannabis preparations. In the same way Navrátil (65) illustrates in his small table of chronic otitis a considerable improvement in 13 of a total of 18 cases. All the cases under investigation were also controlled bacteriologically. In clinical practice the very good results achieved with the preparations mentioned above — i.e., with the alcoholic solution and the dusting powder — stand in support of the good results obtained *in vitro* and justify the suggestion to have both these preparations made available for pharmaceutical production.

The manifold utilization of these substances particularly in the form of alcoholic solutions with glycerine has been confirmed by the results obtained after application of this preparation in *obstetrics* in order to prevent staphylococcic mastitis in the treatment of rhagades and fissures on the nipples of nursing women. Of great importance is the locally anaesthetic component together with the antibacterial effect, particularly upon *Staphylococcus aureus*, which is considered to be the causing agent of the postpartum inflammation of the mamma. Heczko & Krejcí (33) evidenced staphylococcus in 84% of the total of 160 parturient women. It is significant that in 89% of cases the staphylococcus was resistant to penicillin, in 18% to streptomycin, and in some cases resistant to penicillin, streptomycin, aureomycin and terramycin and sensitive only to chloromycetin and to the IRC.

The publication of the results obtained from these investigations evoked a lively interest among obstetricians who up to the present have tried to find means of substitution both for the inadequate and still used gentian violet, and the local inefficacious application of some antibiotics as, for example, aureomycin, which has been recommended by some authors.

For the purpose of *dermatology*, a salve containing 2% of effective substances has been prepared and found very useful in pyodermia of staphylococcic etiology, infected burns, and particularly in decubitus of immobile patients in rehabilitation centres. Of great interest was the follow-up of a physician and pathologist, who was treated with the IRC for a severe infection of the thumb of the right hand, an injury he suffered in the dissecting room. The severe condition, threatening amputation, and the absolute resistance of the microflora to available antibiotics were overcome by substances from cannabis.

The results obtained in treatment of specific *tuberculous fistulas* and published by Proček (70) as well as the up to now unpublished results of the investigations carried out at Vyšné Hágy must be considered as preliminary ones even though the antituberculosis effect *in vitro* is very satisfactory.

In the cases mentioned above, no extraordinary allergy of the organism to effective substances from cannabis or any particular ability of the IRC to sensitize were observed.

In conclusion we may still consider the interesting results Šírek (80) achieved. He mentions his experiences for some years past with hemp seeds in the therapy of *tuberculosis*. The hemp seed was ground and extracted with milk at a temperature of 60-80°C. It represented a protein-rich moiety of the curing diet for tuberculous children, in whom a remarkable improvement due to this diet was observed by the author. But the assumed antituberculotic action of cannabis seeds could not be evidenced in experiments on guinea pigs. Therefore, in the cases reported by Šírek, the therapeutic effect can be only explained by the healing nutritious diet in which the specially prepared seed is of great importance.[8]

In human therapy the best results have been obtained with the following medicaments combined with substances derived from cannabis: dusting powder together with boric acid (otitis), ointment (staphylococcus infected wounds, staphylodermia and so on), ear drops (otitis chron.), alcohol solutions with glycerine (treatment of rhagades on the nipples of nursing women — prevention of staphylococcic mastitis), aqueous emulsions (sinusitis), dentin powder with the IRC (caries). The preparations mentioned above have been already tested clinically, and will eventually be made available for production.

The high yield of effective substances (average 1.5% of the IRC in the dried drug), the considerable amount of raw material available (1.5 million hectares of cannabis plant culture in the world), and the subsequent low price both of the raw material and of the effective substances allow us to utilize the antibacterially effective substances from cannabis in *veterinary medicine*. Such use, which is analogical to human medicine, is comparatively wide: infected wounds (dusting powder), panaris of sheep and cattle (dusting powder), otitis in dogs (ear drops, dusting powder), mastitis in cattle,

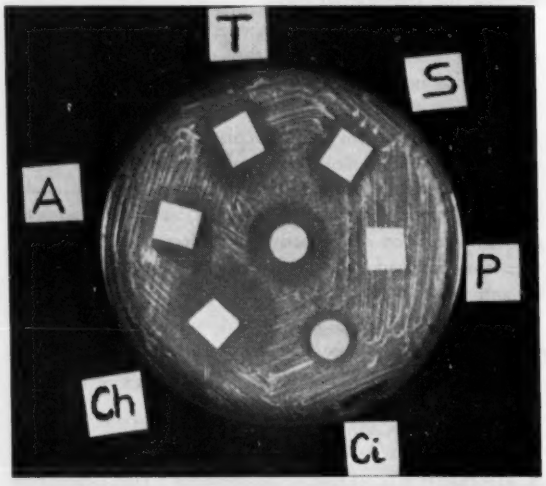


FIGURE 20

Comparison of the inhibitory effect of some antibiotics in combination with active substances from cannabis upon Staphylococcus aureus

P — penicillin 100 OU; S — streptomycin 1 mg; T — terramycin 5 mg; A — aureomycin 5 mg; CH — chloramphenicol 5 mg; Ci — tincture cannabis

Zone in the centre of the plate — 2 per cent of IRC.

[8] A considerably higher resistance against virus pneumonia has been observed in mice fed with hemp seeds.

postpartum trauma, lacerated wounds and so on. Especially veterinary workers, workers concerned with cattle breeding, employees in slaughter houses, cutters and so on consider the preparations containing biologically active substances to be an important contribution to the prevenion of tuberculosis, anthrax, swine erysipelas and brucellosis in various streptococcic and staphylococcic infections of the hands and the like. The results achieved in this field, but unpublished as yet, justify the greatest optimism.

In view of the results obtained with antibacterially effective substances isolated from cannabis, it may be concluded that they are superior and have numerous advantages as antibiotics.

The results obtained in clinical practice must receive, for the most part, a positive evaluation. In many cases satisfactory and in some cases even surprisingly good results have been obtained, particularly where a microflora resistant to a series of other antibiotics was concerned. Naturally drawbacks had to be recorded. Further clinical experiences and practice will be necessary for a definite evaluation of this antibacterially effective substance.

Summary

A systematic investigation of the mid-European flora has been carried out in order to establish the presence of antibacterially effective substances. A high content of remarkably active substances has been found present in *Cannabis sativa var. indica* and in *Cannabis sativa*, a plant cultivated for the industrial utilization of the hemp fibre. It has been investigated in detail from the bacteriological and the chemical viewpoint and from the standpoint of the eventual applicability of the effective substances in clinical practice.

In old herbals and in folk medicine in Europe, Africa and America, mention has been made of the utilization of cannabis as an antibiotic and analgetic medicament particularly for external use. The hashish effect has been known only to nations with a warm climate. In Europe, hashish made its appearance as a narcotic at the time of Napoleon's campaign in Egypt, but contrary to morphine and cocaine, its use was not widespread. In spite of this, from the pharmacological viewpoint, official medicine has investigated the hashish effect on the central nervous system whilst the analgetical and antiseptic effects have been unjustifiably forgotten.

In the chemical section of this work, the most suitable methods of extraction have been described and the technique for the isolation of effective substances developed. At the beginning of the procedure two antibacterially effective fractions were obtained: the acid and the phenolic. Then from the acid fraction a biologically active crystalline acid could be isolated. On the basis of the elementary analysis, the optical rotation, the UV- and the IR-spectroscopy, the following constitution has been established: 3-methyl-6-isopropenyl-4′-n-pentyl-2′-6′-dihydroxy-1, 2, 3, 6-tetrahydrodiphenyl-3′-carboxylic acid. Because of its close relationship to cannabidiol, it has been named cannabidiolic acid. The second-antibacterially ineffective acid has been identified as *trans*-cinnamic acid. In addition, the following unsaponified constituents have been isolated: paraffin (n-nonacosane) and higher alcohols.

Experimentally we could evidence the bactericide effect of the substances from cannabis *in vitro* upon the following gram-positive micro-organisms: *Staphylococcus aureus haemolyt*, *Staphylococcus aureus*-resistant to penicillin, *Streptococcus alpha*, *Streptococcus beta haemolyticus*, *Pneumococcus*, *Enterococcus*, *Corynebacterium diphtheriae*, *Bacillus anthracis*, *Erysipelothrix rhusiopath*. A significant antibacterial effect upon the *Mycobacterium tuberculosis in vitro* could be observed up to a dilution of 1: 150,000.

The gram-negative micro-organisms of the coli-typhus group, as well as the *Pseudomonas aeruginosa*, *Proteus vulg.* and, in addition, the yeast and the fungi, proved to be resistant.

The limit of the effectiveness of the maximal dilution of the biologically active substances (1: 100,000) and the velocity of the effect of various concentrations could be established.

The effect of the inactivating factor has been studied in detail. Blood, plasma and serum partially inactivate and decrease the antibacterial effect.

A comparison between the effectiveness of the active substances from cannabis and that of penicillin and streptomycin has been carried out at various pH.

Finally, the experiments made in clinical practice, particularly in stomatology, otorhinolaryngology, gynecology, dermatology, phthisiology, with some pharmaceutical preparations containing antibacterial substances from cannabis have been reported. Attention has been drawn to the advantageous utilization of the active substances from cannabis in veterinary medicine, and particularly in preventive medicine for anthropozoonoses.

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Only those works referred to in our paper are listed, for at that time the *Bulletin on Narcotics* was not available to us. In the *Bulletin* there are two reports to be found (Chopra, J. C.: vol. *9*, No. 1, 5-32 (1957), and Ahmed Benabud: vol. *9*, No. 4, 1-17 (1957)) which, in discussing the history of the utilization of cannabis in popular medicine in India and Morocco, refer to the analgesic and antibacterial effects of cannabis (erysipelas, dysentery, gonorrheoa, cholera, etc.). Some other papers are particularly concerned with the analysis, the chemistry, and the narcotic effect of cannabis — viz., Duquénois, P.: vol. 2, No. 3, 30 (1950); Bouquet, J.: vol. *2*, No. 4, 14-31 and vol. 3, No. 1, 22-46 (1951); Cordeiro de Farias, R.: vol. 7, No. 2, 6 (1955); Asahina, H.: vol. *9*, No. 4, 18 (1957); Joachimoglu, G.: vol. *11*, No. 3, 5 (1959).

In vol. *3*, No. 1, 62-81 and No. 2, 47-54 (1951) of the above-mentioned journal a comprehensive literature on cannabis and its utilization is given. In the bibliography of the *Bulletin*, there have also been listed the periodical publications on cannabis up to 1958.

Research on various kinds of fungi observed on Turkish opium and their removal with different sorts of fungicide

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Soil Products Office of Turkey, Opium Section

I. — Introduction

It was observed on some occasions that opium purchased in different regions of this country was covered with mould. We therefore proceeded to research with the purpose of determining species of fungi causing moldiness on opium and their action on the opium alkaloids.

The first part of our work on the subject was published in 1958 in the United Nations *Bulletin on Narcotics*, vol. X, No. 4. We then learned from *The Journal of Japanese Botany*, vol. 33, No. 2, February issue, pp. 24-27, that Takashi Matsushima & Haruyo Asahina of the National Hygienic Laboratory, Tokyo, Japan, had been carrying out research on the same subject, results of which were shown in the above journal. These scientists succeeded in isolating a new species which they called Oospora sp., growing on Turkish opium. Thereupon, we reverted to the subject and proceeded to comparative studies on the results obtained by the Japanese scientists.

The second part of our work was reported in 1959 as " Species of fungi isolated from moldy samples of opium from ten different regions ".

During our research we did not find any Oospora sp. as determined by the Japanese scientists on Turkish opium. However, before stating definitely that no Oospora sp. growth was observed on Turkish opium, we thought that research should be first extended to the entire country. As explained hereafter, we carried out our research on 265 samples, which we personally collected, ensuring sterilization, from 75 opium-purchasing agencies in Turkey.

Eleven species of fungi causing mould on opium, spreading over opium-producing areas in this country, were isolated and forwarded to Holland and England for identification. In the meantime, we proceeded to apply different fungicides with varied active elements with the purpose of removing fungi attacking opium.

We would like to express our gratitude to Dr. Haydar Okaner, Assistant General Director of the Turkish Soil Products Office, Ankara, and Mr. Hürrem Balkan, Director of the Opium Department, for their help and for making it possible for us to carry out the work on opium, this product being an important source of income for this country. We further extend our heartfelt thanks to Mr. Selman Açba, Director of the Opium Department, Istanbul, for his interest in our work and his encouragement, and to Prof. Dr. Bekir Alkan of the Ankara University and Doc. Dr. Ibrahim Karaca for their efforts and help on the isolation of fungi. Furthermore we wish to express our deep gratitude to the Commonwealth Mycological Institute, Kew, Surrey, England, and the Centraalbureau voor Schimmelcultures, Baarn, Holland, for their most valuable co-operation in the identification of fungi.

II. — Materials and method

A. — *Materials*

The 265 samples of opium used in our tests were collected by ourselves under required conditions of sterilization, from the 75 opium-purchasing agencies of the Turkish Soil Products Office. The jars in which we placed the opium samples were previously sterilized, and their lids were closed.

Table 1

Origin and number of opium samples

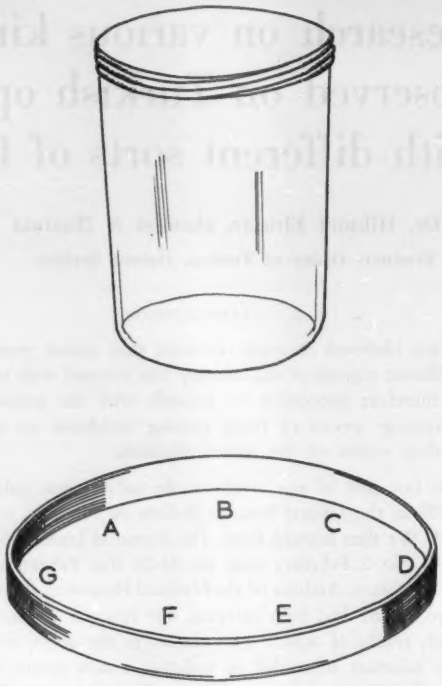
Origin	Province	Number of samples	Dates on which samples were drawn	Collected by
Suhut	Afyon	6	20.VII.59	M. Igneciler
Yildirimkemal	"	4	"	"
Sincanli	"	4	"	"
Emirdag	"	6	21.VII.59	"
Bolvadin	"	6	"	"
Çay	"	6	"	"
Sultandag (Ishakli)	"	4	"	"
Afyon	"	9	22.VII.59	"
Akschir	Konya	8	24.VII.59	"
Sandikli	Afyon	4	25.VII.59	"
Dinar	"	4	"	"
Burdur	Burdur	4	"	"
Korkuteli	Antalya	6	"	"
Tefenni	Burdur	5	27.VII.59	"
Isparta	Isparta	6	28.VII.59	"
Keçiborlu	"	6	"	"
Uluborlu	"	4	"	"
Senirkent	"	4	"	"
Egridir	"	4	29.VII.59	"
Gelendost	"	4	"	"
S. Karaagaç	"	4	"	"
Yalvaç	"	6	"	"
Konya	Konya	4	31.VII.59	"
Karaman	"	5	3.VIII.59	"
Ilgin	"	4	7.VIII.59	"
Argithan	"	5	8.VIII.59	"
Beyschir	"	6	11.VIII.59	"
Seydischir	"	6	"	"
Bozkir	"	5	"	"
Kadinhan	"	4	14.VIII.59	"

TABLE 1 *(continued)*

Origin	Province	Numbes of samples	Dates on which samples were drawn	Collected by
Gümüshaciköy	Amasya	3	27.VII.59	H. Eltutar
Merzifon	„	4	„	„
Çorum	Çorum	1	„	„
Mecitözü	„	4	28.VII.59	„
Amasya	Amasya	5	28.VII.59	„
Erbaa	Tokat	1	29.VII.59	„
Niksar	„	3	„	„
Tokat	„	5	„	„
Zile	„	5	30.VII.59	„
Malatya	Malatya	4	3.VIII.59	„
Nallihan	Ankara	5	5.VIII.59	„
Beypazari	„	5	„	„
Geyve	Kocaeli	1	19.VIII.59	„
Göynük	„	6	„	„
Banaz	Afyon	6	24.VIII.59	„
Usak	Usak	7	„	„
Ulubey	„	2	25.VIII.59	„
Sivasli	„	3	„	„
Simav	Kütahya	9	26.VIII.59	„
Gediz	„	6	„	„
Kütahya	„	2	27.VIII.59	„
Tavsanli	„	9	„	„
Emet	„	6	„	„
Seyitgazi	Eskisehir	4	28.VIII.59	„
Sivrihisar	„	3	„	„
Yunusemre	„	4	„	„
Eskisehir	„	4	„	„
Bilecik	Bilecik	2	29.VIII.59	„
Gölpazari	„	2	„	„
Inegöl	Bursa	4	31.VIII.59	„
Denizli	Denizli	8	6.IX.59	„
Dazkiri	„	3	„	„
Çal	„	6	7.IX.59	„
Acipayam	„	7	„	„
Çivril	„	4	„	„
Aydin	Aydin	2	8.IX.59	„
Kula	Manisa	2	10.IX.59	„
Alasehir	„	3	„	„
Salihli	„	5	„	„
Selendi	„	3	11.IX.59	„
Sindirgi	Balikesir	1	„	„
Akhisar	Manisa	2	12.IX.59	„
Balikesir	Balikesir	2	„	„
Dursunbey	„	5	14.IX.59	„
Orhaneli	Bursa	4	16.IX.59	„

Mouldy lumps were selected among opium purchased by agencies in the above districts; these lumps were cut in the middle and classified according to their appearance. About 20-30 g were taken together with their media from different lumps of opium which had the same colour and aspect, and these were placed in jars (method 1). The jars were closed and isolated with paraffin; then labels were attached to the jars with indication of the origin of the sample, the marks of the locality, the date on which the sample was drawn, and the name of the person who collected the sample.

Fungicides used in tests were supplied by the Ministry of Agriculture General Directorate of Quarantine and the Faculty of Agriculture Plant Protection Chair Laboratory.



B. — *Method*

The medium used for growing fungi was Czapek-Agar with addition of 20% sucrose. After carefully preparing the medium and placing it in petri boxes, fungi from different samples were inoculated to each one separately. Fungi thus inoculated in petri were left to grow in an incubator at 26°C.

In order to test fungicide action on fungi, the products were evenly mixed with Czapek-Agar media with addition of 20% sucrose and placed in petris. Each species of fungi isolated

TABLE 2

Names of fungicides used in tests, names of manufacturers, and active elements

Product	Manufacturer	Percentage of active element
Copramat	Ciba	Ziran, 27.25 Copper, 32.84
Cu - Sul	Neptune	Copper, 10.50 Sulphur, 19.35
Brestan	Hoechst	Triphenyl-sincacetate, 20
Hortexan	Agro-Merck	Lindane, 20 Phenylqueck-silveracetate, 3.20
Formalin	Merck	Formaldehyde

1960 Dr. Hikmet Eltutar - Mustafa İğneciler

from samples was separately introduced in petri boxes containing media with fungicides; these were then placed in an incubator at 26°C, and their action was tested from time to time. Products containing copper as active element were tested twice for each fungus species; the other fungicides were tested ten times.

As for the determination of the dominant characteristics of fungi species with regard to each other, we used petri boxes of 15-18 cm diameter. We poured Czapek-Agar media with 20% sucrose in these, and inoculated isolated fungi at determined distances (method 2).

Growth of the above in an incubator at 26°C was studied. This test was repeated three times.

III. — Species of fungi isolated from samples collected from all opium-purchasing agencies

As explained in the "Materials" section, a laboratory number was given to each sample of mouldy opium with regard to its origin. Fungi drawn from mouldy parts of the above opium were placed in 20% sucrose-added Czapek-Agar medium, and kept in an incubator at 26°C. It was observed that these fungi appeared visibly 3 days after having been planted. Later on, they were taken out of the incubator and left at room temperature (18°C). A week later, their characteristic colours and shapes could be observed. These fungi were then classified according to their colour and shape.

In order to obtain pure cultures from fungi which were believed to be different from the colour and shape standpoint, isolation was repeated several times, and then these were forwarded to England and Holland for identification.

Fungi species growing on Turkish opium

Aspergillus niger van Tieghem
Aspergillus repens Corda Sacc. (Cda) de Bary
Aspergillus flavus Link
Aspergillus wentii Wehmer
Aspergillus ostianus Wehmer
Fusarium solani (Mart.) App. et emend. Sny. et Hans
Fusarium exysporum Schlect
Penicillium granulatum Bain
Penicillium expansum Link
Penicillium cyclopium Westling
Penicillium stoloniferum Thom

Fungi indicated by letters A, B, C, D, E, F and G in table 3 are the following:

A: *Aspergillus niger* van Tieghem.

B: *Aspergillus repens* (Corda) Sacc. (Cda) de Bary.

C: *Penicillium expansum* Link.
Penicillium cyclopium Westling.
Penicillium stoloniferum Thom.
Penicillium granulatum Bain.

D: *Aspergillus flavus* Link.

E: *Fusarium solani* (Mart.) App. et Wr. emend. Sny. et Hans.
Fusarium oxysporum Schlect.

F: *Aspergillus wentii* Wehmer.

G: *Aspergillus ostianus* Wehmer.

As will be noted from table 3, three different kinds of fungus causing mouldiness on opium and spreading over production areas have been found. These are *Aspergillus*, *Penicillium* and *Fusarium*. Among them, the following species were determined:

1. *Aspergillus: Asp. niger* van Tieghem; *Asp. repens* Corda Sacc. (Cda) de Bary; *Asp. flavus* Link; *Asp. wentii* Wehmer; *Asp. ostianus* Wehmer.

2. *Penicillium: P. granulatum* Bain; *P. expansum*, Link; *P. Cyclopium* Westling; *P. stoloniferum* Thom.

3. *Fusarium: F. oxysporum* Schlect.; *F. solani* (Mart.) App. et Wr. emend. Sny. et Hans.

On 265 samples used in tests, Aspergillus was found on 240, Penicillium on 165 and Fusarium on 16. With regard to origin, Aspergillus was found everywhere, Penicillium was fond in 71 districts excluding Çorum, Geyve, Kadinhan and Kütahya; as for Fusarium, it was found in only 12 districts, namely: Yildirimkemal, Sincanli, Çay, Ilgin, Argithan, Egirdir, S. Karaagac, Yalvac, Simav, Gediz, Zile and Denizli.

The spread of fungi species through opium-producing areas is as follows:

	Number of districts
Aspergillus niger	73
Aspergillus repens	55
Aspergillus flavus	27
Aspergillus wentii	12
Aspergillus ostanius	5
Fusarium solani and F. oxysporum	11
4 kinds of Penicillium (as mentioned above)	71

In conclusion, it was observed that Aspergillus niger, Penicillium granulatum, Penicillium expansum, Penicillium cyclopium and Penicillium stoloniferum are widely spread over the Turkish opium-producing areas, whereas Aspergillus wentii, Aspergillus ostianus, Fusarium solani and Fusarium oxysporum are found in small quantities, and that ecological and climatic conditions have no relation with the development of such fungi.

IV. — Removal of isolated fungi with fungicides

Into 100 cm^3 of the medium used in tests, we added 1 g of each fungicide containing a different active element with 3 g of Formalin, and we prepared different kinds of medium with this. After placing the media in petri containers, we inoculated isolate fungus species in each one separately, then left it to grow in an incubator at 26°C together with a blank (same species in media free of fungicide).

As will be noted from table 4, some products had no action on fungi, whilst others showed conclusive and lasting action.

TABLE 3

Species of fungi isolated from samples collected from all opium-purchasing agencies

Origin	District No.	Laboratory No.	Species of isolated fungi
Salihli	2	1	A
	3	2	B
	1	3	A C
	4	4	B C
Seyitgazi	1	5	A C
	3	6	D
	2	7	C D
	4	8	D
Acipayam	1	9	A F
	3	10	A B F
	2	11	A C
	4	12	B
Eskischir	1	13	A
	3	14	A
	2	15	A C
	4	16	B
Akhisar	2	17	A C
	C/2	18	A B C D
	1	19	C
	C/1	20	A D
Sultandag	2	21	A F
	C/2	22	F
	1	23	A B C
	C/1	24	B
Yildirimkemal ...	2	25	A C
	C/1	26	A C
	1	27	A C
	C/2	28	A B C E
Suhut	2	29	A C
	C/2	30	A B
	1	31	C
	C/1	32	A B
Simav	1	33	E F
	3	34	A C D F
	2	35	A D
	4	36	A F
Ilgin	2	37	A D E
	C/2	38	A C
	1	39	A C D
	C/1	40	A C D E
Seydischir	1	41	A C D
	C/1	42	A C
	2	43	A
	C/2	44	B D
Orhaneli	1	45	B C
	3	46	B
	2	47	C
	4	48	B
Amasya	2	49	A C
	3	50	A C F
	1	51	No growth
	4	52	A B F
	5	53	A C
Dinar	1	54	C
	C/1	55	A B
	2	56	A C
	C/A	57	A
Çorum	1	58	A
Geyve	1	59	A
Korkuteli	1	60	C
	C/1	61	A C D
	2	62	C
	C/2	63	A C
Bilecik	2	64	A C
	1	65	A C
Tefenni	2	66	C
	C/2	67	A B C
	1	68	C
	C/1	69	No growth
Erbaa	1	70	C D
Karaman	1	71	A
	C/1	72	A B C
	2	73	A C D
	C/2	74	A C
Argithan	1	75	A C
	C/1	76	A C E
	2	77	A D
	C/2	78	A C
S. Karaagaç	2	79	A C
	C/2	80	B
	1	81	A C E
	C/1	82	A B E
Yalvaç	1	83	C D
	C/1	84	No growth
	2	85	B C
	C/2	86	A C E
Konya	1	87	C
	C/2	88	B
	2	89	A C
	C/1	90	B

TABLE 3 *(continued)*

Origin	District No.	Laboratory No.	Species of isolated fungi
Senirkent	1	91	A B C
	C/1	92	A B
	2	93	C
	C/2	94	A B
Gelendost	1	95	A C
	C/1	96	A C
	2	97	C
	C/2	98	A B C
Uluborlu	1	99	A B
	C/1	100	A B C
	2	101	B C
	C/2	102	A B C
Keçiborlu	1	103	A B C
	C/1	104	A C
	2	105	C
	C/2	106	A C
Mecitözü	2	107	C
	4	108	A C
	1	109	C
	3	110	B
Sincanli	2	111	A C D E
	C/2	112	A C D
	1	113	A C D
	C/1	114	A C E
Nallihan	2	115	C F
	3	116	A F
	1	117	A F
	4	118	A F
Niksar	2	119	A C
	3	120	B
	1	121	A C
Dazkiri	1	122	A C
	3	123	A B
	2	124	A B C
Beypazari	1	125	A B C D
	3	126	A B
	2	127	A
	4	128	A
Merzifon	2	129	A C
	3	130	A B C D
	1	131	A C
	4	132	A B
Emet	2	133	A B C F
	3	134	A C F
	1	135	A B C F
	4	136	A F
Gediz	2	137	A B C
	3	138	A D
	1	139	A E
	4	140	B
Sivasli	1	141	A
	3	142	A C G
	2	143	A C
Inegöl	1	144	A C
	3	145	A C
Inegöl	2	146	A B C
	4	147	A
Beyşehir	1	148	A B C
	C/1	149	A B C
	2	150	A C
	C/2	151	A F
Tavasanli	2	152	A C
	3	153	A B
	1	154	A C
Kula	2	155	A C
	1	156	A C
Aksehir	1	157	A B C
	3	158	A C
	2	159	A B C
Isparta	1	160	A B C
	C/1	161	B
	2	162	A C
	C/2	163	A C
Gümüşhaciköy ..	2	164	A C
	3	165	D
	1	166	C
Selendi	2	167	A C
	3	168	A C D
	1	169	A C
Emirdag	2	170	A C F
	C/1	171	A B F
	1	172	A C F
	C/2	173	A F
Zile	1	174	B C D
	4	175	A E
	2	176	B C
	3	177	A B C

TABLE 3 *(conclusion)*

Origin	District No.	Laboratory No.	Species of isolated fungi
Bolvadin	2	171	A D
	C/2	179	A C D
	1	180	A B
	C/1	181	A C D G
Göynük	2	182	B C
	3	183	No growth
	1	184	C
	4	185	A B
Çay	2	186	A B C F
	C/2	187	A B C F
	1	188	A C
	C/1	189	A E F
Tokat	1	190	A B
	3	191	B
	2	192	A B C
	4	193	A D G
Malatya	1	194	B C
	3	195	A D
	2	196	D
	4	197	A D
Burdur	1	198	A C
	C/1	199	B C
	2	200	A
	C/2	201	No growth
Egirdir	2	202	C
	C/2	203	A C
	1	204	A C
	C/1	205	E
Banaz	1	206	A C
	3	207	A B C
	2	208	A C
	4	209	A C
Balikesir	1	210	A B C
	2	211	C
Dursunbey	2	212	A C
	4	213	A F
	1	214	A B C
	3	215	A C D
Afyon	1	216	A B C
	C/1	217	A C
	2	218	A C
	C/2	219	A F
Usak	1	220	A B C
	3	221	A D
	2	222	A B C
	4	223	B
Alasehir	1	224	A C
	2	225	A B
Çivril	1	226	A B C
	2	227	A B C
	3	228	A B C
	4	229	A B C
Yunusemre	2	230	A C
	4	231	B
	1	232	C
	3	233	A B C
Sivrihisar	1	234	C
	3	235	A C G
	2	236	A C
Ulubey	1	237	A B C
	2	238	A
Sandikli	1	239	A D
	C/1	240	A C
	2	241	A
	C/2	242	C D
Bozkir	2	243	A
	C/2	244	A C
	1	245	A B C
	C/2	246	A C G
Aydin	2	247	A
	1	248	A C
Çal	1	249	A
	3	250	A B
	2	251	A C
	4	252	A C
Denizli	1	253	A C E
	3	254	A C D
	2	255	A
	4	256	A B C E
Gölpazari	1	257	A B C
	2	258	No growth
Kadinhan	1	259	D
	C/1	260	A D
	2	261	A
	C/2	262	A D
Kütahya	1	263	A
	2	264	A B
Sindirgi	1	265	A C D

TABLE 4

Number of days elapsed before growth of fungi in artificial media with fungicide

Name of product	Fungus mark	Number of days														
		1	2	3	4	5	6	7	8	... 24	25	26	27	28	... 60
Hortexan, 1 g	A	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	B	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	D	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	E	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	F	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	G	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Blank		—	—	—	+	+	+	+	+	+	+	+	+	+	+
Cu-Sul, 1 g	A	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	B	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	C	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	D	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	E	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	F	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	G	—	—	—	—	+	+	+	+	+	+	+	+	+	+
Blank		—	—	—	+	+	+	+	+	+	+	+	+	+	+
Coprantol, 1 g	A	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	B	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	C	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	D	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	E	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	F	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	G	—	—	—	—	+	+	+	+	+	+	+	+	+	+
Blank		—	—	—	+	+	+	+	+	+	+	+	+	+	+
Copromat, 1 g	A	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	B	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	C	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	D	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	E	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	F	—	—	—	—	+	+	+	+	+	+	+	+	+	+
	G	—	—	—	—	+	+	+	+	+	+	+	+	+	+
Blank		—	—	—	+	+	+	+	+	+	+	+	+	+	+
Brestan, 1 g	A	—	—	—	—	—	—	—	—	—	—	+	+	+	+
	B	—	—	—	—	—	—	—	—	—	+	+	+	+	+
	C	—	—	—	—	—	—	—	—	—	+	+	+	+	+
	D	—	—	—	—	—	—	—	—	+	+	+	+	+	+
	E	—	—	—	—	—	—	—	+	+	+	+	+	+	+
	F	—	—	—	—	—	—	—	+	+	+	+	+	+	+
	G	—	—	—	—	—	—	—	—	—	+	+	+	+	+
Blank		—	—	—	+	+	+	+	+	+	+	+	+	+	+
Formalin, 3 g	A	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	B	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	D	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	E	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	F	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	G	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Blank		—	—	+	+	+	+	+	+	+	+	+	+	+	+

The sign (—) in the above table signifies that there was no growth of fungus. The sign (+) signifies that growth had begun. The capital letters stand for fungus species as indicated in table 3. As will be noted from the table, blanks developed visibly 3 days after being planted. All fungi in media with Cu-Sul, Coprantol and Copromat which contain copper began to grow on the fifth day following the date on which they were planted.

In media with Brestan, Aspergillus wentii, Fusarium appeared on the 8th day; Aspergillus flavus the 24th day; Aspergillus repens, Aspergillus ostianus and all Penicillium species appeared on the 25th day; Aspergillus niger developed

on the 26th day. No species planted in media containing Hortexan and Formalin developed within 60 days. Cu-Sul, Coprantol, Copramat and Brestan had no complete action on fungi and we therefore had to be content with these tests.

As for Hortexan and Formalin, these products showed conclusive and lasting action, and research was continued using the above fungicides in order to determine their minimum action dosage. The results are shown in table 5.

TABLE 5

Minimum doses of fungicides showing conclusive action

Name and dose of product	Fungus mark	Number of days															
		1	2	3	4	5	6	7	8	...	24	25	26	27	28	...	60
Hortexan, 0.500 g	A	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	B	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	C	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	D	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	E	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	F	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	G	—	—	—	—	—	—	—	—		—	—	—	—	—		—
Blank		—	—	—	+	+	+	+	+		+	+	+	+	+	+	+
Hortexan, 0.250 g	A	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	B	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	C	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	D	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	E	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	F	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	G	—	—	—	—	—	—	—	—		—	—	—	—	—		—
Blank		—	—	—	+	+	+	+	+		+	+	+	+	+		+
Formalin, 2 g	A	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	B	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	C	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	D	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	E	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	F	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	G	—	—	—	—	—	—	—	—		—	—	—	—	—		—
Blank		—	—	—	+	+	+	+	+		+	+	+	+	+		+
Formalin, 1 g	A	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	B	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	C	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	D	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	E	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	F	—	—	—	—	—	—	—	—		—	—	—	—	—		—
	G	—	—	—	—	—	—	—	—		—	—	—	—	—		—
Blank		—	—	—	+	+	+	+	+		+	+	+	+	+		+

It will be observed from the above table that Hortexan and Formalin showed conclusive action even with minimum doses. On basis of these results obtained in artificial media, tests will be carried out on natural media (opium).

V. — Study of the dominant characteristics of fungi

Research was carried out with the purpose of determining whether fungi causing mouldiness on opium showed any predominance over each other under equal conditions regarding medium, temperature and culture.

In tests carried out on the subject, as described in the " Materials " section of this report, 20% sucrose added Czapek-Agar was placed in large petri containers of 15-18 cm diameter, then each species of fungi was planted at equal distances using a fine needle. Fungi thus inoculated developed in three days at 25°C and colonies covered entirely the petri surface within a week. No marked difference was observed on the spreading area of fungi in the petri containers.

VI. — Conclusions

1. — Fungi species causing mouldiness on opium throughout opium-producing regions of Turkey are the following:

Aspergillus niger van Tieghem
Aspergillus repens Corda Sacc. (Cda) de Bary
Aspergillus flavus Link
Aspergillus wentii Wehmer

Aspergillus ostianus Wehmer
Fusarium solani (Mart.) App. et Wr. emend. Sny. et Hans.
Fusarium oxysporum Schlect.
Penicillium granulation Bain
Penicillium expansum Link
Penicillium Cyclopium Westling
Penicillium stoloniferum Thom

2. — Spread of the above species in this country is shown in table 4.

3. — Fungicides which gave the best results for the removal of fungi were Hortexan and Formalin.

VII. — Literature

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SCIENTIFIC PUBLICATIONS ON NARCOTIC DRUGS IN 1959

Introduction

The following bibliography has been prepared by the secretariat, and includes scientific publications on narcotic drugs which appeared during 1959, as well as a few which appeared earlier but could not, for various technical reasons, be included in the preceding lists published in the *Bulletin on Narcotics*. This bibliography makes no claim to be complete, and information regarding books or articles not included in it would be gratefully received by the Division of Narcotic Drugs, European Office of the United Nations, Geneva, Switzerland.

The bibliography has been arranged by subject in the following way:

Part. I. General works

Part II. Opium poppy, opium and drugs manufactured therefrom

- A. Opium poppy, opium and opium alkaloids collectively considered
- B. Morphine
- C. Other phenanthrene alkaloids and derivatives of opium
- D. Other drugs and substances derived from the opium poppy

Part III. The coca leaf and cocaine

Part IV. Cannabis and its derivatives

Part V. Synthetic drugs

- A. General works
- B. Pethidine and related compounds
- C. Methadone and related compounds
- D. Morphinan and morphine synthesis

Part VI. Addiction to narcotic drugs

Part VII. Miscellaneous

Under each heading, publications are arranged alphabetically by author. Owing to the nature of the sources available to the secretariat for preparing this bibliography, several titles are given in translation only. They are preceded by an asterisk.

The bibliography is prepared for both English and French editions; therefore translations in both languages follow those titles which are not in one of the working languages.

Part I. — GENERAL WORKS — GÉNÉRALITÉS

A

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Part II. — OPIUM POPPY, OPIUM AND DRUGS MANUFACTURED THEREFROM
PAVOT A OPIUM, OPIUM ET DROGUES QUI EN SONT TIRÉES

A. Opium poppy, opium and opium alkaloids collectively considered
Pavot à opium, opium et alcaloïdes de l'opium considérés dans leur ensemble

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ERRATUM

Volume XII, No. 1

Article entitled " Physico-chemical methods for the identification of narcotics ", by Kl. Genest & C. G. Farmilo.

p. 19, left-hand column:

line 28: Delete the word " sprayed ".

line 29: Delete the word " dihydrogen ".

line 30: Delete the word " phosphate ".

Table V: Delete from the heading the words " phosphate-treated papers ".

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Commission on Narcotic Drugs. REPORT OF THE FOURTEENTH SESSION, 27 APRIL - 15 MAY 1959, ECONOMIC AND SOCIAL COUNCIL, OFFICIAL RECORDS, TWENTY-EIGHTH SESSION, SUPPLEMENT No. 9. English, French and Spanish editions published annually . . . . . *Price per issue:* $0.50; 3/6 stg. Sw. Fr. 2.—

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